Corrigenda

P 109 line 7 Insert after *Puccinia glumarum* (Schm.) Erikss. & Henn. (syn. *P. striiformis* West.)

"Accurate measurement and control of environmental variables is basic to elucidation of the role of physical elements of the environment in development of plant disease. Such information is an integral part of epidemiology, upon which rests the ultimate goal of phytopathologists - the control of plant disease."

Dimock, A. W. (1967)

Annual Review of Phytopathology 5 : 265-284.

EFFECT OF VARIATION IN HOST, PATHOGEN AND ENVIRONMENTAL FACTORS ON LEAF RUST IN POPLAR CAUSED BY *MELAMPSORA MEDUSAE* THÜM.

By

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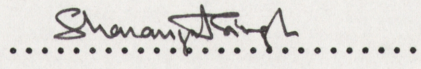
STATEMENT OF ORIGINALITY

Except where acknowledged this thesis is my own original work.



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Sharan P. Singh

ABTRACT

Employing a detached leaf culture technique the reactions of cultivars of *Populus* species to *Melampsora medusae* Thüm were studied under different controlled environmental conditions. Replicate leaf disks ($1.76cm^2$) of various cultivars were inoculated with urediniospores of *M. medusae*, placed on plastic foam soaked with 10 p.p.m. gibberellic acid and subsequently incubated at various environmental conditions. The host/pathogen reactions were assessed employing a qualitative (basis infection type) or a quantitative (basis various disease parameters; time from inoculation to flecking and eruption of uredinia, uredinial density and amounts of urediniospores produced) scale.

When 21 cultivars of *Populus* species, raised under controlled conditions in a rust-free glass-house, were screened for susceptibility to a field collection of *M. medusae*, cultivar constitution was a very significant (P = 0.001) determinant of disease level, assessed on various parameters. Incubation period and latent period were highly correlated (R^2 = 0.73, P = 0.005) but these two parameters were poorly correlated with the uredinial number.

Six mono-urediniospore isolates of *M. medusae*, produced qualitatively distinct reactions when incubated at 15°C on leaf disks of certain cultivars of *P. deltoides* Marsh. and thus were recognised as distinct pathogenic races. The infection type in some race-cultivar combinations were very temperature sensitive with less distinct reactions when incubated at 20 or 25°C.

Infection types were assesed when four cultivars of *P. deltoides*

were inoculated with six races of *M. medusae* and incubated in combinations of light intensity (100, 500, 1000 μE m^{-2} s^{-1}) and temperature (15, 25°C). Increasing light intensity was generally associated with reduced infection type (i.e. apparently higher resistance) in cultivars; the reduction was more pronounced at an incubation temperature of 25 than 15°C.

The effects of combinations of post-inoculation temperature (15, 25°C) and light intensity (100, 500, 1000 μE m^{-2} s^{-1}) on disease severity (three parameters), induced by six races of *M. medusae* in four cultivars of *Populus* species were assessed in a factorial experiment. While all cultivars were immune when incubated at 25°C/1000 μE m^{-2} s^{-1}, most cultivars were susceptible, to varying degrees at all other temperature/light intensity combinations. Although the relative rating for disease severity of race/cultivar combinations depended to a degree on the parameter employed, the temperature of incubation, irrespective of the parameter used, was the most important determinant of variation in severity. All major variables, and their two- and three-way interactions, were significant determinants of variation in the number of uredinia developed per unit area. The ranking of the cultivars for relative resistance, and of races for relative aggressiveness, depended on the temperature/light intensity combination during incubation.

Replicate clones of compatible cultivars of *Populus* species, grown for three months on a high (28/20°C - day/night) temperature regime and incubated, following inoculation with a mixture of urediniospores of six races of *M. medusae*, on a high (28/20°C - light/dark) temperature regime, were more resistant (as determined by

uredinial number) to leaf rust than those raised and incubated on any other combination of high (28/20°C) and low (20/10°C) pre-/post-inoculation temperature regimes. Irrespective of the the parameter employed to assess disease severity, cultivar constitution, pre- and post-inoculation temperature were significant contributors to the level of disease induced in the cultivars by *M. medusae*. The rating of cultivars for relative resistance was somewhat dependant on the parameter employed to assess disease and for most parameters there were significant interactions of cultivar, pre-, and post-inoculation temperature in determining the disease level induced. The coefficient of determination between parameters of disease severity were low.

Disease severity (six parameters) was assessed on leaf disks, cut from four cultivars of *Populus* species, raised on either long (15 h) or short (10 h) photoperiod, inoculated with urediniospores of race 4 of *M. medusae*, and subsequently incubated on either long (15 h) or short (10 h) photoperiod. While disease severity, based on most parameters, was greater in disks from the continuing long (15 h pre- and 15 h post-inoculation) than from the continuing short (10 h pre- and 10 h post-inoculation) photoperiod, maximum severity was developed by leaf disks in the combination of short pre- and long post-inoculation photoperiod. The relative contribution of the cultivar, the pre- and the post-inoculation photoperiod and their second and third order interactions to variation in disease severity depended on the parameter employed to assess severity.

Four cultivars of *Populus* species were inoculated with urediniospores of race 4 of *M. medusae* and subsequently incubated at combinations of temperature (15, 25°C), light intensity (100, 400 μE

m^{-2} s^{-1}) and photoperiod (10, 15 h) regimes. Disease severity as assesed on most of the six parameters employed was generally higher in the combination of of 25°C, 100 μE m^{-2} s^{-1} and short or long photoperiod of incubation than the remaining temperature/light intensity/photoperiod treatments. The analysis of variance demonstrated that most of the major factors (cultivar, temperature, light intensity and photoperiod) and their interactions were significant causes of variation in various parameters of disease severity.

To sum up, the results have demonstrated a range of variability in the resistance/susceptibility of cultivars of *Populus* species to *M. medusae* leaf rust and in the virulence/aggressiveness in the races of *M. medusae*, and the specificity (qualitatively differential interaction or quantitatively significant race/cultivar interaction in the ANOVA) of race/cultivar reactions. The epidemilogical significance of these host/pathogen relationships is discussed. Further, the reactions of certain race/cultivar combinations are differentially sensitive to the environmental factors. The role of such sensitivity in maintaining disease stability, under field situations, is discussed.

PUBLICATIONS BY THE AUTHOUR FROM THIS THESIS

Singh, S. J. and Heather, W. A. (1981). An improved method for detached leaf culture of *Melampsora* leaf rust of *Populus* species. ***Transactions of the British Mycological Society*** **77** : 436-437.

Singh, S. J. and Heather, W. A. (1982a). Assessment *in vitro* of resistance in cultivars of *Populus* to *Melampsora medusae* Thüm. Leaf rust. ***Australian Forest Research*** **12** : 37-45.

Singh, S. J. and Heather, W. A. (1982b). Temperature sensitivity of qualitative race-cultivar interactions in *Melampsora medusae* Thüm. and *Populus* species. ***European Journal of Forest Pathology*** **12** : 123-127.

Singh, S. J. and Heather, W. A. (1982c). Temperature sensitivity of light inhibition of uredospore germination in *Melampsora medusae*. ***Mycologia*** **74** : 472-478.

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Singh, S. J. and Heather, W. A. (1983c). Sensitivity to pre- and post-inoculation temperature of the reactions of *Populus* spp. to *Melampsora medusae* Thüm. ***Forest Ecology and Management*** (In press).

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CHAPTER 1

INTRODUCTION

1.1 SIGNIFICANCE OF THE GENUS *POPULUS*

Various species of *Populus* (Salicaceae) occur naturally and are grown commercially over a wide range of the northern hemisphere (Anon., 1979). A brief description of the taxonomic divisions of the genus is given in Appendix 1a. Cultivation of poplars is recommended by the F.A.O. to meet the expected heavy timber requirements of the increasing world population (Anon., 1958). Poplars can be easily vegetatively propagated, they have a phenomenal growth rate and produce high yields of good quality light wood with diversity of uses. For example, poplar wood is well suited to industries which manufacture fibre boards, plywood, paper pulp, light box making materials and safety matches etc. (Anon., 1979). Some species of *Populus* are important also in wind breaks, erosion control (Anon., 1958; van Kraayenoord, 1968) and ornamental plantings (Heather and J. K. Sharma, 1977). Poplar leaves are rich in nutrients and, in certain parts of the world, are used as fodder for the cattle (Anon., 1979). The use of poplars in combination with cattle grazing as a form of agro-forestry has been advocated in eastern Australia by Mr. Keith Lober (Wilson, 1981).

1.2 PROSPECTUS OF POPLARS IN AUSTRALIA

Poplars were introduced into the southern hemisphere initially as ornamentals and subsequently commercial plantations were established. Poplars were brought into Australia in the mid nineteenth century (Hall and Brown, 1958; Palmberg, 1977) but first commercial plantations were established in 1960's when the desirability of poplar wood for safety match production (Brown, 1971) and the prospectus for its use in the plywood manufacture (Pryor, 1976) provided necessary stimulus. The silvicultural role of poplars in Australia has been summarized by Pryor (1969). The estimated area under poplar cultivation in Australia in 1976 was *ca.* 6000 hactares, mainly in the New South Wales and Victoria (Anon., 1976) and there has been only a marginal increase in area under poplar cultivation in the past six years. Commercial poplar plantations can be established only on land with good agricultural potential hence, the possibility of extension of area under poplar cultivation depends on the economic relationship of different forms of use of good quality agricultural land on one hand and the competition with wood from pine plantations on the other hand. Breeding of poplar cultivars with high productivity potential, e.g., traits like insensitivity to day-length and resistance to diseases could possibly result in an increase in area under poplar cultivation (Pryor and Willing, 1965). In Australia, pines are fulfilling most of the demands for general purpose wood but for several reasons, e.g., diversity of wood supplies and safety measures against disease epidemics, the cultivation of other fast-growing genera in appropriate regions is desirable (Hillis and Brown, 1978).

1.3 DISEASES OF POPLARS

Poplars are susceptible to a number of non-parasitic (caused by climatic disturbances, e.g., wind, frost, cold, sun-light etc.; unfavourable soil, and toxic substances) and pathogenic (caused by fungi, bacteria, viruses, nematodes and parasitic phanerogams) diseases (Anon., 1979). Diseases of economic importance in poplar are caused by an array of micro-organisms, e.g., bronze leaf (*Marsonina brunnea* (Ell. *et* Ev.) P. Magn.), leaf rust (*Melampsora* species) Septoria leaf spot (*Septoria populi* Desm.) and cankers (*Septoria musiva* Peck., *Hypoxylon prunatum* (Klotche) Cke., *Xanthomonas populi* Ride (Anon., 1979).

1.4 LEAF RUST OF *POPULUS* SPECIES

Leaf rust, the most important disease of poplars in many situations, is caused by a number of species of *Melampsora* (*Melampsoracae, Uredinales, Basidiomycetes*) and occurs wherever poplars grow. Certain variations in occurrence (Appendix 2a) and severity of these rust species occur in different geographic locations (Anon., 1979). The presence of *Melampsora* leaf rust on poplars is readily recognised by the appearance of uredinia (Plate 1), which produce powdery masses of bright orange to yellow urediniospores, abundantly and sometimes exclusively on the abaxial surface of the infected leaves. Telia appear late in the summer as slightly raised areas or crusts which are initially orange yellow but later become dark or black. All the *Melampsora* spp. causing leaf rust of poplars, except *M. aecidioides*

Plate 1 A young plant of *P. X euramericana* cv. *I-488* bearing uredinia (yellow pustules) of *M. medusae* on its leaves.

on *Populus alba* L. (van Vloten, 1944; Arthur and Cummins, 1962), are heteroecious (i.e. require an alternate host species to complete their life-cycle) and macro-cyclic (i.e. produce all well known types of spores typical of a rust fungus, e.g., aecio-, uredinio-, teleuto-, basidio-, and pycniospores) (Arthur and Cummins, 1962). The life-cycle of a typical macro-cyclic *Melampsora* causing leaf rust of poplar is described in Appendix 3a.

The species of *Melampsora* are identified generally according to the shape and size of different types of spores (usually urediniospores) formed in their life-cycle, spore walls (Arthur, 1934; Fresa, 1941; Hennerbert, 1964; Taris, 1968; Pinon, 1973; Walker *et al.*, 1974; Taris *et al.*, 1977), paraphyses (Gremmen, 1954; Milatović and Šarić, 1966; Pinon, 1973; Walker *et al.*, 1974) and their host range (Arthur and Cummins, 1962; Ziller, 1965). Some controversy exists over the taxonomic grouping of certain species of *Melampsora* (van Vloten, 1944; Ziller, 1965; Steenackers, 1969). Physiologic races within certain species, e.g., *Melampsora laricipopulina* Kleb. (van Vloten, 1944; J. K. Sharma and Heather, 1976a; Miller, 1978; Chandrashekar and Heather, 1980; Latch and Wilkinson, 1980) and *Melampsora medusae* Thüm (J. K. Sharma and Heather, 1976a) based on their reactions on differential cultivars of *Populus* species are known to occur.

1.5 DAMAGE CAUSED BY LEAF RUSTS

Severe leaf rust incidence causes pre-mature senescence and early defoliation which results in poor growth and even die-back of poplar

seedlings and trees (Nagel, 1949; Peace, 1952, 1958, 1962; van der Meiden and van Vloten, 1959; Toole, 1967; Anon., 1971; Schipper and Dawson, 1974; van Kraayenoord, 1974; Walker *et al.*, 1974; Siwecki, 1976). The *Melampsora* leaf rust is generally more damaging in young rather than in old trees (J. K. Sharma *et al.*, 1980). The rust pustules disrupt the leaf epidermis and the infected foliage suffers from increased transpiration loss (Schipper, 1976a). Successive heavy infections of newly formed leaves during a particular growing season cause severe defoliation and prevent the maturing of over-wintering buds. Such buds produce shoots which are abnormally susceptible to frost-injury (Peace, 1952). Seriously affected plants become more susceptible to other diseases, e.g., cankers caused by *Cytospora* species and *Dothichiza* species (Schreiner, 1951; Peace, 1952; van der Meiden and van Vloten, 1959). The exact details on the losses of yield resulting from the attack on poplars by particular species of *Melampsora* are lacking. However, a decrease of 10 to 20 per cent in effective growing season, due to leaf rust infection of poplars, is reported (Leontovyc, 1958). A moderate attack of rusts can cause 46 per cent reduction in volume growth of poplar trees and thus is a serious threat to maximum fibre production (Schipper and Dawson, 1974; Widin and Schipper, 1976, 1981). Leaf rust incidence which causes pre-mature defoliation in ornamental poplars reduces their aesthetic value (Heather and J. K. Sharma, 1977).

1.6 POPLAR LEAF RUST IN AUSTRALASIA

Following their introduction into Australia, *M. medusae* of northern

American origin and *M. larici-populina* of European origin, caused leaf rust epidemics on poplars in an area near Sydney in 1972 and 1973, respectively (Walker and Hartigan, 1972; Walker *et al.*, 1974). From January to April 1972, *M. medusae* leaf rust had spread 400 kilometers north into Queensland and more than 600 kilometers into Victoria (Marks and Walker, 1972). The rate of spread of initial outbreaks (1972) of *M. medusae* leaf rust is illustrated in Appendix 4. Some susceptible cultivars of poplar e.g. *Populus deltoides* Marsh. cv. *angulata* which, beacuse of their high productivity, had been extensively planted in eastern Australia, were seriously damaged by this rust species and subsequently many plants of this cultivar died (Walker *et al.*, 1974). *M. larici-populina* was also observed first near Sydney and later found in Victoria and Queensland mainly on ornamental plantations of *Populus nigra* L., while some cultivars of *P. X euramericana* (Dode) Guinier were susceptible also (Walker *et al.*, 1974). Following their introduction and epidemic appearance in Australia these rusts were observed in New Zealand (McMillan, 1973; van Kraayenoord, 1974; Wilkinson and Spiers, 1976). The distribution of the two rust species in Australasia is summarized in Appendix 2b.

1.7 POPLAR LEAF RUST RESEARCH IN AUSTRALIA

A collection of cultivars of poplar which were introduced into Australia from different provences in the United States of America (Brown, 1971) showed a wide spectrum of reactions, e.g., complete susceptibility to immunity against *M. medusae* and generally a high degree of resistance against *M. larici-populina* (Eldridge *et al.*,

1973). The great diversity of the resistance in the cultivars combined with the ease of intra- and inter-specific hybridization (Appendix 1b), the availability of resistant cultivars for commercial plantations by clonal propagation and the feasibility of mass production of resistant hybrid progenies is promising in combating the leaf rust epidemics. However, the possibility of rapid production of physiologic forms by these rust species with virulence on the selected resistant cultivars (J. K. Sharma and Heather, 1976a) necessitates a continuous programme of selection and breeding of poplars for rust resistance.

The initial outbreak of *M. medusae* leaf rust in southeastern Australia was more serious than that of *M. larici-populina* leaf rust. However, in the following years (post 1974) the severity of *M. medusae* in Canberra area has declined and *M. larici-populina* is the major leaf rust species in this area. In contrast, *M. medusae* is the predominant leaf rust species in the northern New South Wales (Kempsey, Grafton, etc.). Because of greater relative importance of *M. larici-populina* in Canberra area, the initial investigations at the Australian National University, Canberra, concentrated on this pathogen (Heather, personal communication). The major research involved screening of cultivars of poplar against this rust pathogen (J. K. Sharma and Heather, 1976b), recognition of physiologic races based on their qualitative and quantitative reactions on differential cultivars of poplar (J. K. Sharma and Heather, 1976a; Miller, 1978; Heather *et al.*, 1980a,b; Chandrashekar and Heather, 1980), testing the response of race/cultivar interactions at variable levels of certain factors of environment, e.g., temperature (Chandrashekar and

Heather, 1981a), light intensity (Chandrashekar and Heather, 1981b) and temperature/light intensity combinations (Chandrashekar and Heather, 1982), and the pre-disposing effects of temperature (Chandrashekar and Heather, 1981c). A series of experiments on the interaction of certain hyperparasites, mainly species of *Cladosporium* with the leaf rust development to investigate the possibility of biological control of rust have been conducted (J. K. Sharma and Heather, 1978, 1980; Omar and Heather, 1979; I. K. Sharma and Heather, 1982). The host/pathogen reactions were highly sensitive to the factors of the physical (Heather and Chandrashekar, 1982, 1983) and biological (I. K. Sharma and Heather, 1982) environment in which the interaction occurred. This demonstrated the highly interactive model of this host/pathogen/environment system and the consequent problems in understanding the epidemiology of *M. larici-populina* leaf rust of poplar. Similar investigations on *M. medusae* leaf rust of poplar were desirable to establish both similarities and contrasts with *M. larici-populina*.

1.8 PUBLISHED INFORMATION ON *M. MEDUSAE* LEAF RUST OF POPLAR

M. medusae was first described in the U.S.A. by Thuemen (1878). A detailed description of characteristics of different types of spores produced in its life-cycle is given in Appendix 3b. This rust caused severe defoliation of poplars in the lower Mississippi River Valley (Toole, 1967) and Rhinelander, Wisconsin (Schipper and Dawson, 1974). Thielges and Adams (1975) reported higher losses in increment of rust susceptible cultivars as compared with rust resistant cultivars. In

the north-central United States, Widin and Schipper (1981) reported that *M. medusae* caused losses from 29 to 32 per cent in poplars in dry weight and 31 to 42 per cent for wood volume.

While *M. medusae* is endemic to north America it has extended its geographic distribution following its introduction into France, Europe (Dupias, 1943) and Australasia (Walker and Hartigan, 1972; Heather and J. K. Sharma, 1977). The distribution of this rust species in different countries is described in Appendix 2b. The introduction of this pathogen into new areas, previously free of this organism and which had plantations of poplar cultivars, not selected for resistance against *M. medusae*, has caused considerable losses. Its introduction into Australia and killing of susceptible widely planted cultivars, e.g., *P. deltoides* cv. *angulata* is a classical example.

The Aigeiros and Tacamahaca sections of poplars are the normal hosts of *M. medusae* but recently its pathogenicity, under laboratory conditions on *P. alba* of section Albidae, generally considered immune to this rust and suitable for use in breeding rust resistant interspecific hybrids was reported (J. K. Sharma and Heather, 1977). *M. medusae* also occurs on black and white hybrid poplars and section Turanga (R. Willing, personal communication). An extensive investigation of its alternate host species incorporating a number of previously unreported species was conducted by Ziller (1965). Some information on its poplar and alternate host species is given in Appendix 2a and 2c.

1.9 THE NEED FOR *IN VITRO* STUDIES ON *M. MEDUSAE* LEAF RUST

In the field in Australia, *M. medusae* occurs in the same areas and on some of the same hosts as *M. larici-populina*. In fact, the two species occasionally occur on the same leaf. In U.S.A., *M. medusae* is associated generally with *Melampsora aecidioides* (D. C.) Schroet. and *Melamspora albertensis* Arth. In such situations it may be possible to select poplar cultivars with a broad range of resistance against more than one species of *Melampsora*. However, precise epidemiological studies of *M. medusae* leaf rust in such field situations are not possible. In addition, poplar hosts and *M. medusae* have an extensive latitudinal distribution, e.g., *ca.* 47°N in U.S.A. to 38°S in Australasia (Heather, personal communication). Thus investigations, employing controlled laboratory physical environmental variables (Nelson and Kline, 1963; Dimock, 1967; Hooker, 1967), pure cultures of the *Melampsora* species and cloned plants of the cultivars of the poplar are necessary to understand the fundamentals of host/pathogen/environment relationships. The preliminary information from the laboratory studies have a significant value, particularly in a high-labour-cost country like Australia, in planning efficient programmes for research under the field situation.

1.10 THE AIMS OF THE STUDIES REPORTED IN THIS THESIS

1 Investigate the spectrum of resistance/susceptible reactions in cultivars of poplar to *M. medusae* leaf rust.

2 Standardise a set of differential cultivars of popalr and to investigate the occurrence of physiologic races in *M. medusae*.

3 Investigate the effects of certain pre- and post-inoculation environmental factors (e.g. temperature, light intensity and photoperiod) on *Populus* (cultivars)/*M. medusae* (races) interactions.

4 Based on *in vitro* studies to conceive possible problems and progress in understanding the epidemiology of this leaf rust in the field.

5 Compare similarities and contrasts of host/pathogen/environment relationships for this rust with those of *M. larici-populina* leaf rust of poplar.

These features (1 to 5) will be considered in relation to current knowledge and hypotheses of relationships in plant host/pathogen systems.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

The materials and methods common to two or more experiments reported in following chapters are described here to avoid repetition. Changes in the general procedures and procedures specific to a particular experiment are mentioned in appropriate individual chapters. The suitability of the techniques employed, in comparison with other existing alternatives, has been discussed by Omar (1978) and Chandrashekar (1981) when investigating development of *M. laricipopulina* leaf rust of *Populus* species. Many of the methods used in the present study have been described elsewhere (Omar and Heather, 1975, 1978; J. K. Sharma and Heather, 1976a,b, 1977, 1979a,b; Omar, 1978; Miller, 1978; J. K. Sharma *et al.*, 1980; Chandrashekar and Heather, 1980, 1981a,b,c, 1982; Chandrashekar, 1981; Singh and Heather, 1981, 1982 a,b,c, 1983a,b,c,d) hence, a detailed review of relevant techniques is not given in this thesis.

2.2 SOURCE AND MAINTENANCE OF CULTIVARS OF *POPULUS* SPECIES

2.2.1 SOURCE: Shoot cuttings of cultivars of *Populus* species were obtained from the poplar nurseries of the Botany Department, The

Australian National University, Canberra and of the Division of Forest Research, CSIRO, Canberra. Information on the origin and history of the cultivars is supplied in Appendix 1c and Chapter 3 (section 3.2.2.1; Table 3.1 and 3.4).

2.2.2 PROPAGATION OF PLANTS: The cultivars of poplar were vegetatively propagated as replicated cuttings i.e. clones. In March-April 1980, dormant shoots of cultivars of *Populus* species grown in the field were collected and 10cm long cuttings of uniform thickness (1 to 1.5cm diam.) each bearing 2 to 3 buds were prepared. These cuttings were disinfected by dipping in one per cent sodium hypochlorite solution for two minutes and subsequently washed twice with distilled water. This treatment aimed at elimination of surface fungal spores, including those of *Melampsora* spp., likely to be present on the cuttings. Distal ends of the cuttings were dipped in a harmone preparation (Lane Plant Cutting Powder, 0.05g/kg indole acetic acid, 0.02g/kg napthalene, Roche-Magg Limited, Australia) to stimulate rooting while the proximal ends were sealed with wax to avoid loss of moisture and possible invasion through wounds by wood-rot fungi. The distal ends were then inserted into fully swollen (previously soaked in water) individual Jiffy-7 700® peat pellets (Jiffy Products Limited, Grorud, Norway). The cuttings were kept at 20±1°C in a growth chamber with *ca.* 70 per cent relative humidity regulated by an automatic sprinkling system. After four weeks, when most of the buds had sprouted, these cuttings were transplanted into 6 inch (diam.) plastic pots containing equal proportions of Vermiculite and Perlite (Australian Gypsum, Australia) and

subsequently maintained in a rust-proofed glass-house (double door, wet-pad-filter air inlet) at 15/25°C (night/day). The glass-house plants received a photoperiod (natural light supplemented with artificial fluorescent light) of 16h per 24h. Considerable seasonal fluctuations in temperature and light intensity occurred over the three year period of experimentation. Depending on the temperature and light intensity in the glass-house, the plants were watered (inside the pots), once or twice daily. The pots drained freely (except in summer when saucers were placed underneath the pots) on benches (wire grate tops). Osmocote (Sierra Chemical Company, U.S.A.), a slow-release fertilizer (N:P:K, 18:2.6:10), was applied bi-monthly at the rate of 5g per pot. Irrigation with 300 p.p.m. Aquasol (Hortico Private Limited, Australia; chemical composition presented in Appendix 5) was given twice a week in the intervening periods. Plants grown in the glass-house were very healthy (Plate 2.1). The plants were regularly (60 to 80 days intervals depending on growth rate) cut-back, leaving stumps with 2 to 3 buds.

2.2.3 RAISING PLANTS IN GROWTH CABINETS: Temperature and light affect considerably the growth of poplar; further the age of leaves and the maturity of the shoots on which these are borne reportedly affect the quantitative reactions of cultivars of *Populus* spp. to *M. larici-populina* (J. K. Sharma *et al.*, 1980). Therefore, plants intended for use in quantitative studies of rust development were grown in phytotron cabinets (Morse and Evans, 1962) to minimise possible effects of minor variations in temperature and light (temperature in particular). The plants grown in the glass-house were

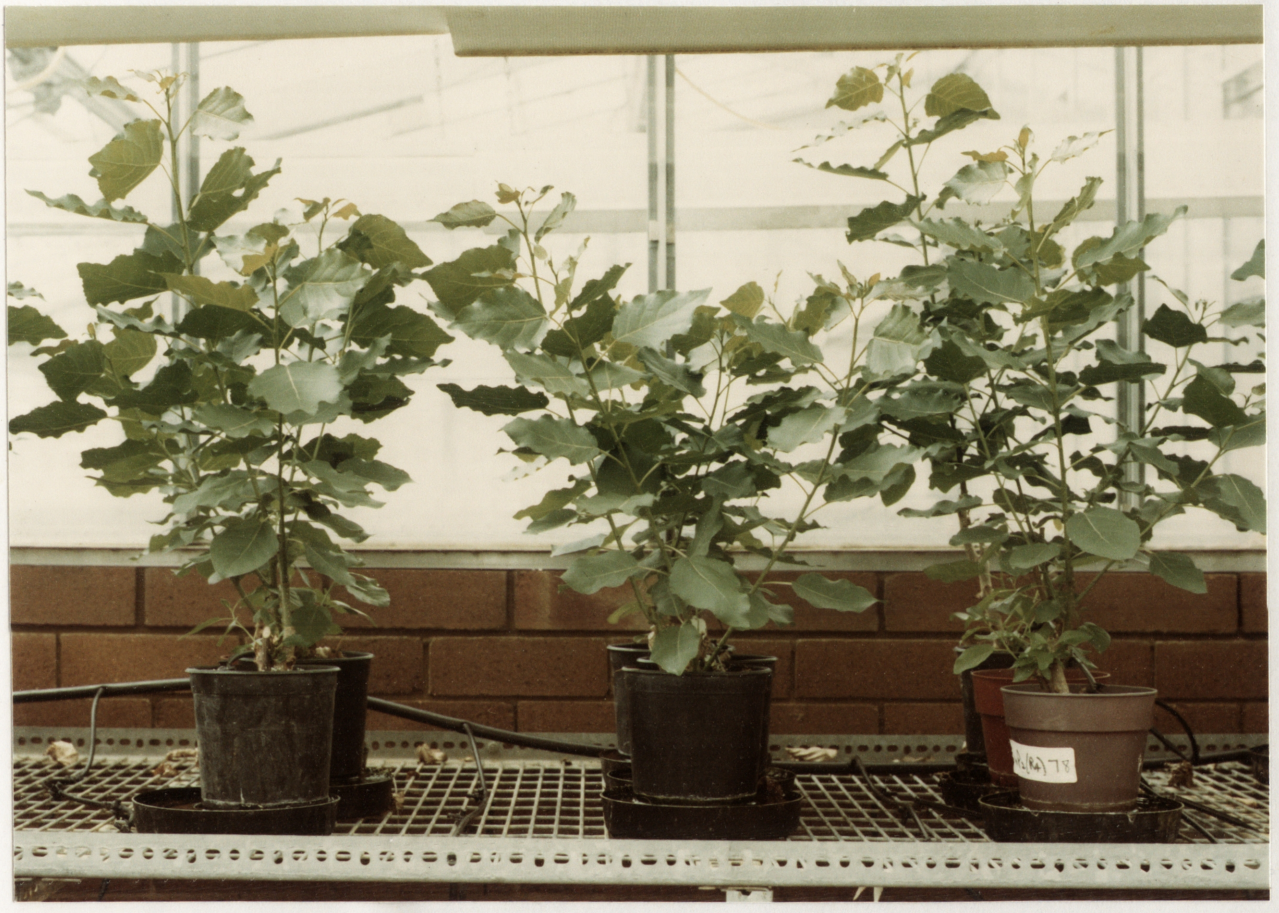

Plate 2.1 Healthy plants of *P. X euramericana* cv. *I-488* growing in the glass-house.

cut-back (section 2.2.2, *ibid.*) and thereafter maintained in phytotron cabinets with closely controlled conditions. The plants in the cabinets (and also in the glass-house) were regularly rotated to to take care of minor differences in the micro-climate (Mastalerz, 1977). The plants grown in cabinets were irrigated with Hoagland solution (Appendix 6) in the morning (10 to 11 a.m.) and with tap water in the evening (3 to 4 p.m.).

2.2.4 PLANT PROTECTION: In the glass-house, infestation by whiteflies, caterpillars and especially mites was occasionally serious while in the growth cabinets these could be easily controlled by proper sanitation measures. The glass-house grown plants were sprayed with Metasystox [3ml/litre of water; active ingredient: 25 per cent (w/v) Demeton-S-Methyl, Bayer Australia Limited] or Kelthane [5ml/litre of water; active ingredient: 24 per cent (w/v) Dicofol, Hortico (Australia) Private Limited]. The frequency of spraying depended on the severity of the insect-pests. Spraying with these chemicals had some toxic effect, resulting in limited leaf necrosis on young plants less than four weeks old. Phytotoxicity and residual toxicity of these chemicals to the leaves used in experiments was minimised by spraying the plants only when necessary and collecting the leaf samples for inoculation at least two weeks after the last spraying. Plants grown in the cabinets were not sprayed with chemicals.

2.3 SELECTION AND PREPARATION OF LEAVES AND LEAF DISKS FOR INOCULATION:

2.3.1 SELECTION OF LEAVES OF UNIFORM AGE AND MATURITY: The position of the leaves on a shoot (age) and the maturity of the shoot on which these are borne have a significant effect on their susceptibility to *M. larici-populina* (J. K. Sharma *et al.*, 1980). Therefore, for all the experiments, a uniform procedure in which leaves were selected only from the central part of shoots of comparable maturity, was followed. Leaf maturity (time from leaf initiation) varied between the experiments upto a maximum of 15 days because of practical problems involved in syncronizing the availability of leaves of a particular maturity and fresh urediniospore inoculum. Within individual experiments the maturity of the leaves sampled from the cultivars varied by only 2 to 3 days.

2.3.2 TREATMENT OF LEAF SURFACE: Despite the care taken in raising the plants either in glass-house or growth cabinets, occasional infestation of leaves by insects (section 2.2.4, *ibid.*) and saprophytic fungi occurred. Certain saprophytic phylloplane fungi are reported to hinder the development of *M. larici-populina* (section 1.7, Chapter 1) hence the leaves were surface sterilized prior to inoculation with urediniospores. Satisfactory surface sterilization was achieved by immersing detached leaves in one per cent sodium hypochlorite for one minute. The leaves were subsequently washed in three changes of sterilized distilled water and dried between folds of sterilized blotter sheets. When necessary the treated leaves were kept briefly in polythene bags in a regrigerator before use in the experiments.

2.3.3 PREPARATION OF LEAF DISKS: Employing a sterilized cork-borer (Appendix 7) leaf disks (1.76 cm^2) were cut from the surface treated (section 2.3.2, *ibid.*) leaves avoiding major veins. These leaf disks were collected in polythene bags and mixed thoroughly to ensure randomisation. Leaf disks were used immediately, or stored briefly in a refrigerator before using in the inoculation experiments.

2.4 SOURCE OF UREDINIOSPORES

2.4.1 COLLECTION OF UREDINIOSPORES FROM THE FIELD: In March 1980, leaves bearing uredinia of different cultivars were collected in the nursery of the Botany Department of The Australian National University. Leaves of each cultivar were collected in individual polythene bags and transported to the laboratory in an ice-box. Urediniospores from the surface of these leaves were removed with a vacuum spore collecting device and the leaves were surface treated (section 2.3.2, *ibid.*). Subsequently, the leaves were floated on 10 p.p.m. gibberellic acid solution in Petri plates and incubated at 20±1°C and 100 $\mu E\ m^{-2}s^{-1}$ in an incubator (Plate 2.2). This treatment ensured that the new crop of urediniospores produced was free of contaminants. The identity of the spores produced on a particular cultivar is important in the studies involving use of pure inoculum of races or species of *Melampsora*. Urediniospores developed on the leaves of different cultivars were collected separately employing a spore collecting device. These were dried *in vacuo* at 20±1°C over silica gel for 24h followed by another 24h over phosphorus pentoxide

Plate 2.2 Incubation chambers (a), having uniform light intensity of 100 μE m^{-2} s^{-1}, installed in a temperature (20±1 °C) controlled room.

and stored in air-tight plastic vials. Thereafter, these plastic vials were sealed in sterile glass jars containing silica gel and stored at -16°C before the spores were used in further inoculations. Low moisture content prolonged the viability of the urediniospores (Shain, 1979). These spores were used to produce fresh batch of urediniospores for use in the subsequent experiments.

2.4.2 PREPARATION OF MONO-UREDINIOSPORE ISOLATES: Spores selected from a single uredinium were dusted on a glass slide and a single urediniospore was selected using a fine tipped glass needle (prepared by stretching a Pasteur pipette over hot flame) under a binocular microscope. A surface treated (section 2.3.2, *ibid.*) leaf segment of *P. X euramericana* cv. *I-488* was inoculated with this urediniospore. This segment was incubated (section 2.4.1, *ibid.*) for 14 days and urediniospores from the newly emerged uredinium were harvested and used in subsequent inoculation. Thus a series of collection and inoculation was required to obtain desired quantity of inoculum. The same procedure was repeated with other selected urediniospores to produce a collection of mono-urediniospore isolates. The virulence (Chapter 4) and aggressiveness (Chapter 5) of the mono-urediniospore isolates were compared on a number of cultivars of *Populus* species. Information regarding the source (i.e. cultivar from which a particular mono-urediniospore isolate was developed) is given in Appendix 3c.

2.5 INOCULATION OF LEAVES AND LEAF DISKS WITH UREDINIOSPORES

2.5.1 INOCULATION OF LEAVES: Surface treated (section 2.3.2, *ibid.*) leaves or leaf segments of *P. X euramericana* cv. *I-488* cut to fit within 10cm (diam.) Petri plates were used for general multiplication of urediniospores. The abaxial surface of the leaves was inoculated uniformly with urediniospores employing a sterilized brush. The results of this inoculation procedure are illustrated in Plate 2.3, showing a uniform distribution of uredinia on a leaf which was inoculated as above and incubated at 20±1°C for 10 days as described (section 2.4.1, *ibid.*). The procedures of collecting, drying and storage of urediniospores have been described elsewhere (section, 2.4.1, *ibid.*).

2.5.2 INOCULATION OF LEAF DISKS: Leaf disks (section 2.3.3, *ibid.*) were arranged randomly on the revolving base of a spore settling tower (described in next section 2.7). A weighted quantity, usually 5mg of dried urediniospores, was placed in the spore gun and blown into the perspex chamber employing an air-current of 70 Kpa for 30 seconds. The sliding lid at the base of the tower was closed for the first 10 seconds while the spores were being dispersed to collect clumps of spores, and removed thereafter. The removal of clumps improved the uniformity of urediniospore deposition on the leaf disks and eliminated possible effects of self-inhibition or self-stimulation (Bell and Daly, 1962). Following the 30 seconds release period most of the spores settled on the leaf disks within 5 minutes. (If some cover glasses were placed in the tower after this 5 minutes, no spores were recorded on these). Thereafter, the leaf disks were recovered from the tower for incubation at particular treatments.

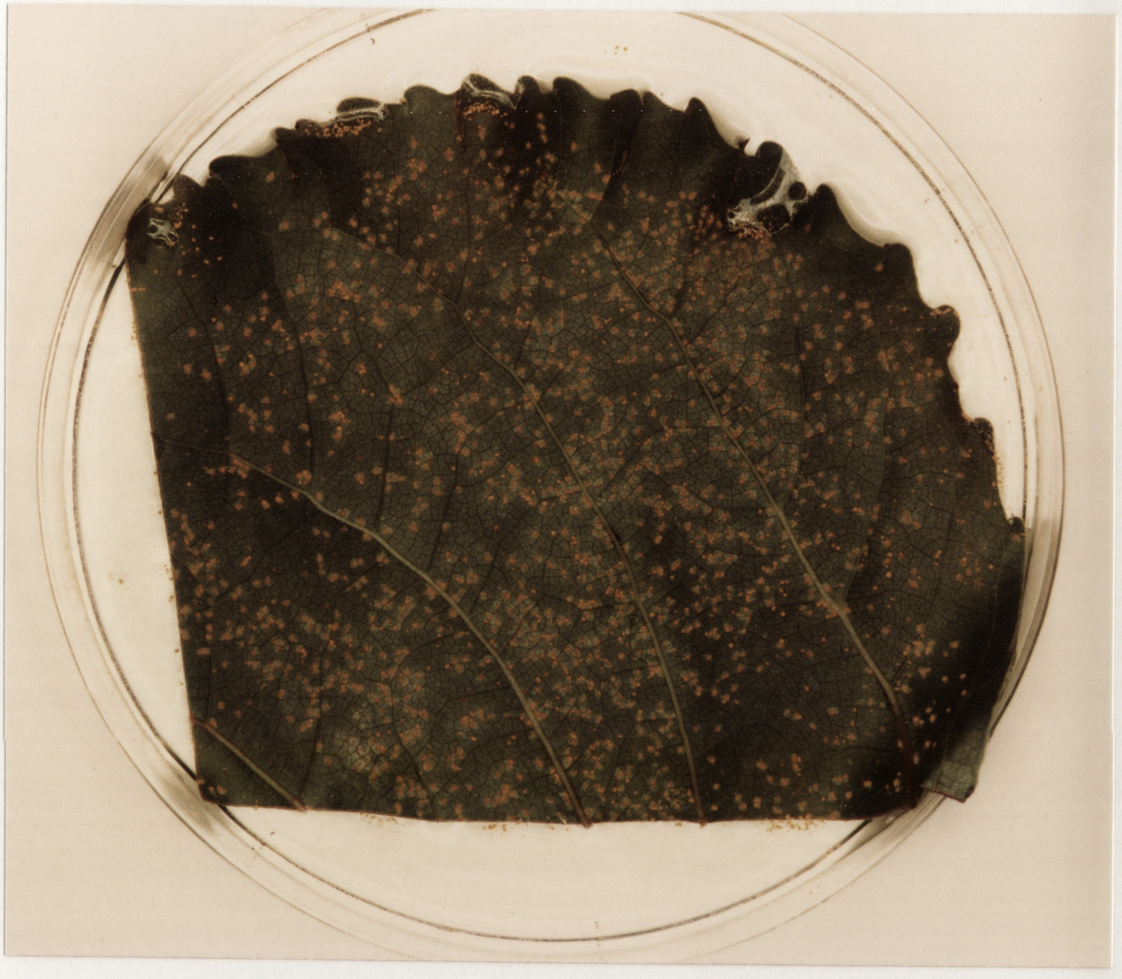

Plate 2.3 A demonstration of uniform distribution of uredinia emerging from a leaf segment of *P. X euramericana* cv. *I-488* inocualted with urediniospores employing a brush and incubated at 20±1°C/100 μE m^{-2} s^{-1} (photographed at 10 days after inoculation).

This uniform inoculation procedure was followed throughout the experiments.

2.6 INCUBATION OF LEAVES AND LEAF DISKS

2.6.1 LEAVES: Inoculated leaves used for mass multiplication of urediniospores were floated on 10 p.p.m. (mg/litre) gibberellic acid solution in Petri plates incubated at 20±1°C and 100 μE $m^{-2}s^{-1}$.

2.6.2 INCUBATION OF LEAF DISKS: Leaf disks were placed on plastic foam thoroughly soaked with 10 p.p.m. gibberellic acid solution in divided 14cm (diam.) glass Petri plates (Plate 2.4) and incubated in the selected environmental conditions. The advantages of this method, especially in quantitative studies on the development of rusts on leaf disks, are described elsewhere (Appendix 7 and section 9.2, Chapter 9).

2.7 SPORE SETTLING TOWER:

Numerous methods of inoculation of host leaves are reported in literature. Brushing (Russel, 1976), swabbing (Mount and Ellingboe, 1968; Smith, 1969), spraying, and dusting with dry spores are some of the common methods. In most studies, especially in quantitative studies on rusts, a settling tower has been used to achieve uniform deposition of spores (Peterson, 1959; Manners and Hossain, 1963; Tollenaar and Houston, 1966a; Eversmeyer and Burleigh, 1968; Yirgou and Cadwell, 1968; Maddison and Manners, 1972; Brown and Kochman, 1973; Omar and Heather, 1975; J. K. Sharma *et al.* 1980)

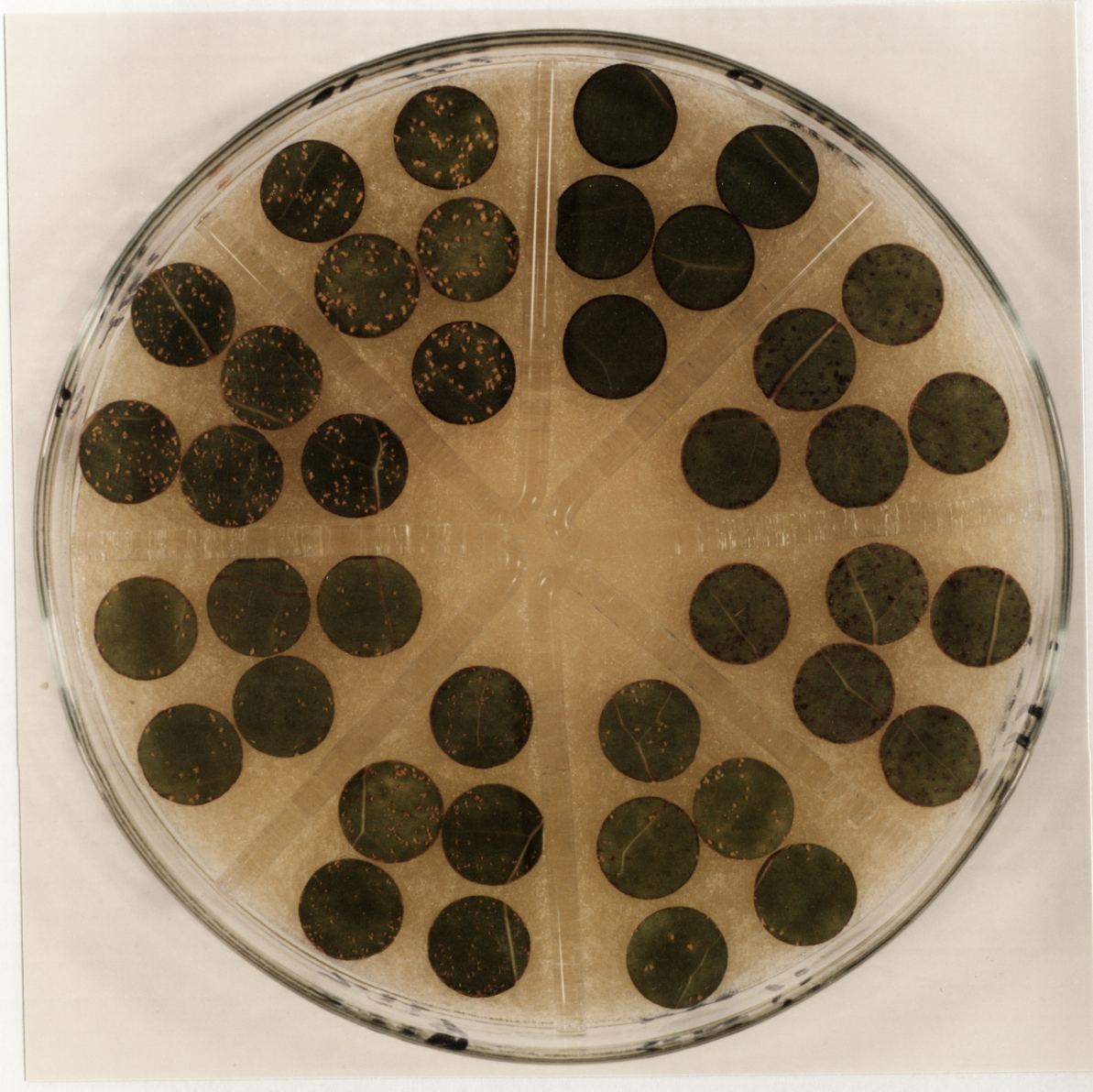

Plate 2.4 A divided 14cm (diam.) Petri plate containing leaf disks of different cultivars inoculated with urediniospores of *M. medusae* and placed on plastic foam soaked in 10 p.p.m. gibberellic acid solution.

A spore settling tower was reported as satisfactory for inoculation of poplar leaf disks with urediniospores of *Melampsora* species in routine studies on host/pathogen relationships. In the present study, a spore settling tower (Figure 2.1) designed and costructed by Dr. M. B. Omar, formerly a Ph. D. student of this department was employed. Two modification were made in the tower. Firstly, a millipore filter was installed between the air-pump and spore gun to ensure that air supply did not contaminate the urediniospores (Appendix 7). Secondly, a revolving base (3 r.p.m.) , replaced the original stationary base; this produced more uniform deposition of urediniospores. These modifications in the tower are illustrated (Plate 2.5).

The tower (inside dimensions of 23.5 X 23.5 X 116.5cm length, breadth and height respectively) was made of perspex. The design of the tower is illustrated (Figure 2.1). Preliminary studies were conducted to determine, the optimum weight of urediniospore inoculum to achieve satisfactory infection frequency of the leaf disk of the cultivars employed and the acceptable number of replicate leaf disks, based on variation in the density of urediniospores deposited. The results of preliminary investigations are summarised in Appendix 8. The inoculation procedure has been described (section 2.5.2, *ibid.*).

2.8 ASSESSMENT OF DISEASE SEVERITY

2.8.1 QUALITATIVE REACTIONS (INFECTION TYPES): Since their original description by Stakman and Levine (1922), infection types based on the disease symptoms have been widely used to characterise races of

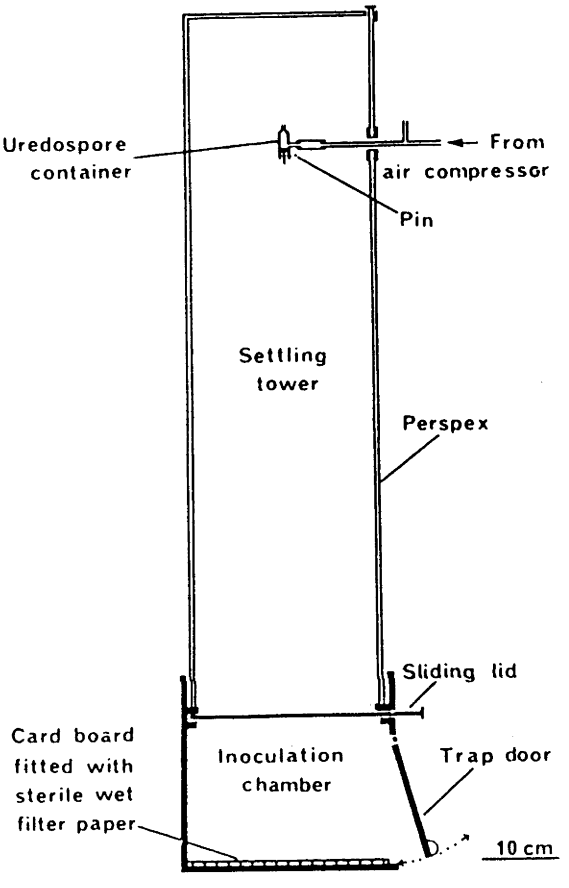

Figure 2.1 Diagram of the spore settling tower employed to inoculate leaf disks of poplar with urediniospores of *M. medusae* (Reproduced from Sharma *et al.*, 1980).

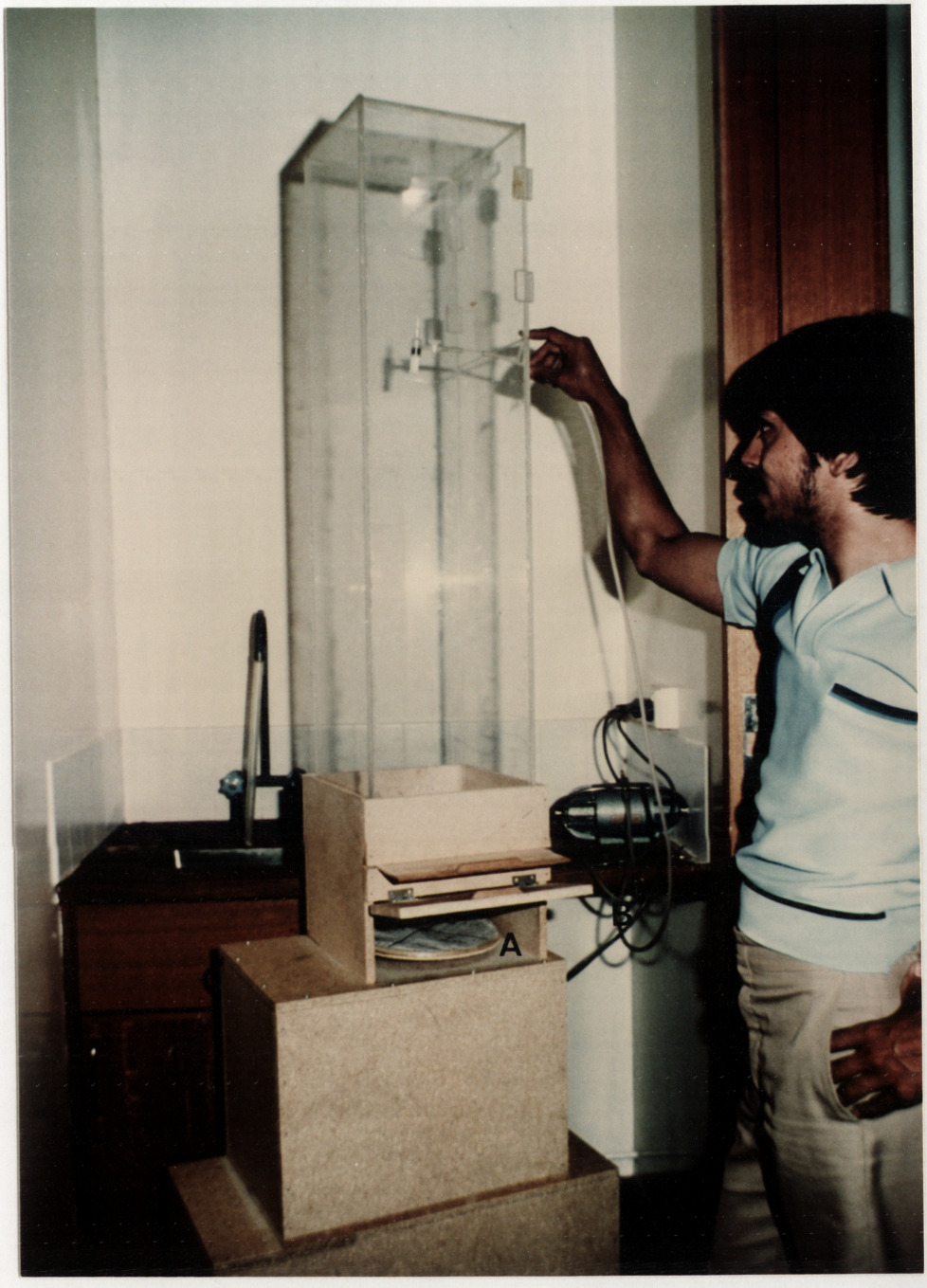

Plate 2.5 The modified spore settling tower. A, revolving base; B. millipore filter.

the pathogens and differential cultivars of host plants. In the present studies a modified version of the qualitative scale used by Singh and Sokhi (1980) to describe the infection types in pea (*Pisum sativum* L.) and lentil (*Lens esculentus* L.) rust (*Uromyces viciae-fabae* Schroet.) was adopted. The various infection types employed were: 0 = Immune - no macroscopic symptoms; 1 = Highly resistant - Necrotic and/or chlorotic flecks; 2 = Resistant - Small uredinia surrounded by necrotic areas; 3 = Susceptible - Small uredinia mixed with necrotic flecks; 4 = Highly susceptible - a few to many uredinia showing copious sporulation; X = Intermediate - 1+2+3+4.

2.8.2 QUANTITATIVE REACTIONS: Disease severity was assessed during a mono-cycle which involves several components viz., incubation period, latent period, uredinial eruption and development. The relevant literature contains conflicting terms to define these different processes (Teng and Close, 1978) hence a description of the terms used here is considered necessary. The different processes in a disease mono-cycle (from inoculation to maturity of uredinia) are illustrated (Figure 2.2).

2.8.2.1 INCUBATION PERIOD (DAYS) TO FLECKING (IPF): Time between inoculation (ta, Figure 2.2) and the appearance of flecks (td, Figure 2.2); flecks are localised chlorotic or necrotic areas, the earliest visible symptoms of disease. Time to fleck production was recorded on individual leaf disks in a particular treatment and the mean value was calculated.

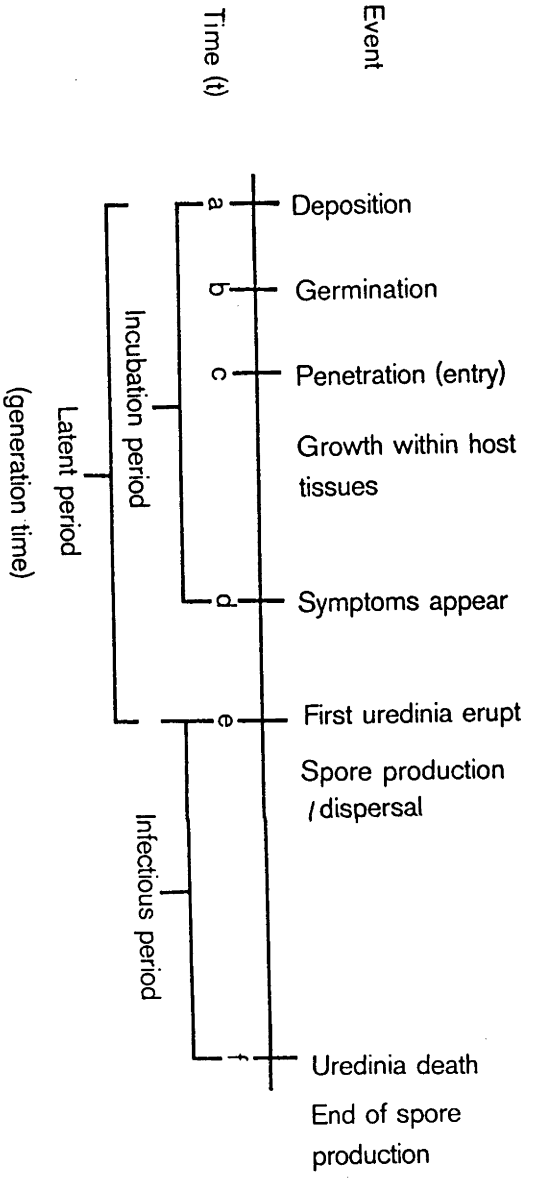

Figure 2.2 Diagramatic representation of a typical mono-cycle of a rust (Reproduced from Teng and Close, 1978).

2.8.2.2 LATENT PERIOD (DAYS) TO ERUPTION OF THE FIRST UREDINIUM (LP1): Time from inoculation (ta, Figure 2.2) to the appearance of the first uredinium (te, Figure 2.2). Mean LP1 was calculated from observations on individual replicate leaf disks in a particular treatment.

2.8.2.3 LATENT PERIOD (DAYS) TO APPEARANCE OF 50 PER CENT UREDINIA (LP50): Time from inoculation (ta) to the production of 50 per cent uredinia recorded at the termination of a particular experiment (somewhere between te and tf, Figure 2.2). Number of uredinia produced per leaf disk was recorded daily and LP50 was calculated from the cumulative data. For a particular treatment the mean LP50 values were calculated from observations on individual replicate leaf disks.

2.8.2.4 NUMBER OF UREDINIA PER LEAF DISK (ULD): Refers to number of uredinia produced per leaf disk at the termination of a particular experiment. Daily counts of the number of uredinia produced per leaf disk were made and the experiments were terminated when this number ceased to increase. The cumulative total uredinia was employed because observations in the middle of an epidemic might not truly indicate the relative resistance/susceptibility of cultivars in terms of ULD.

2.8.2.5 NUMBER OF UREDINIOSPORES PER MM^2 (USM): A measure of sporulation which has potential importance in epidemiological studies (Johnson and Taylor, 1976; J. K. Sharma and Heather, 1979a,b). The

value depends on the density of uredinia (i.e. the ability of the fungus to colonise the host tissue) and the number of urediniospores produced per uredinium (fecundity). The estimation of USM is described (next section 2.8.2.8).

2.8.2.6 NUMBER OF UREDINIOSPORES PER UREDINIUM (USU): This parameter measures the fecundity of a pathogen. As with USM, USU was estimated (as described in following section 2.8.2.9) at the termination of an experiment and hence is cumulative value dependant on size (age) of the uredinium.

2.8.2.7 ESTIMATION OF USM AND USU: These two parameters were estimated after the final count of number of uredinia per leaf disk. Batches of five randomly selected leaf disks from a particular treatment were transferred into sterile McCartney bottles containing 5ml of sterilized water-agar solution (one per cent (w/v) Difco agar). This solution contained Tween-20 (polyoxy ethylene sorbitan monolaurate, ICI Americas Inc. Wilington DE), a wetting agent, at the rate of two drops per litre. Urediniospores were dislodged into the solution by agitating the bottles vigorously on a mechanical shaker for 45 minutes. Haemocytometer (Neubauer counting chamber) counts on the resulting suspension were made to estimate the number of urediniospores in the suspension (Sheridan, 1976) by using the following formula:

$$\text{Number of spores/ml} = \frac{\text{Number of spores in 4 corner squares}}{\text{4 (No. of squares)}} \times 100{,}000$$

2.8.2.8 ESTIMATION OF USM: From the above equation (section 2.8.2.7, *ibid.*) the number of urediniospores produced on five leaf disks (= number of urediniospores in 5ml solution) was calculated. USM was calculated as follows:

$$USM = \frac{\text{Number of spores in 5ml solution}}{\text{5 X 176 (=leaf area used in preparing 5ml solution)}}$$

Mean USM was calculated from the replicate bottles for each treatment.

2.8.2.9 ESTIMATION OF USU: A record of total number of uredinia on the five leaf disks in each bottle was kept and USU was calculated as follows:

$$USU = \frac{\text{Number of spores in 5ml solution}}{\text{Number of corresponding uredinia}}$$

Replications in a particular treatment were used to calculate mean USU.

2.8.2.10. CHOICE OF PARAMETERS: The number of parameters employed and the duration of a mono-cycle in different experiments varied (specific information is provided in the following individual chapters). In experiments involving large number of leaf disks the most time consuming parameters, e.g., USM and USU were not recorded. Additionally, in factorial experiments the developmental stage of

uredinia at a particular time depended on the race, cultivar, and the levels of the factors of pre-conditioning and post-inoculation incubation environment hence there were obvious complications (Teng and Close, 1978) in interpreting the results of USM and USU.

2.9 PRECAUTIONS FOR ASEPTIC CONDITIONS

A number of phylloplane fungi (Bier, 1965; Magnani, 1967, 1970; Morelet and Pinon, 1973; Shain, 1976; section 1.7, Chapter 1) and bacteria (Vasil'ev *et al.*, 1970) are known to contaminate the developing uredinia of *Melampsora* species causing leaf rust of *Populus* species. Similar micro-organisms can affect differentially the reaction (especially quantitative) of poplar cultivars to races of *Melampsora* species (Heather and Chandrashekar, 1983). Thus aseptic conditions were employed particularly in experiments involving a number of cultivars and pure cultures of races, the reactions of which could be differentially sensitive to damage caused by these contaminants. Hence, the leaves were surface treated and dried with sterilized blotting paper, leaf disks were cut with a sterilized cork borer, leaves were inoculated with a sterilized brush, leaf disks were inoculated in a sterilized spore settling tower located in a sterile chamber, while sterilized distilled water was used for preparation of gibberellic acid solution. Assessment of disease development, in closed Petri plates, was made in a sterile chamber. These steps helped to overcome the danger of differential sensitivity (Heather and Chandrashekar, 1983) of race/cultivar reactions to hyperparasites.

2.10 STATISTICAL ANALYSIS OF DATA

The quantitative data on various parameters of disease assessment were analysed using suitable statistical tests. The basic assumptions of analysis of variance (ANOVA), normality of distribution of errors and homogeneity of the variance (homoscedasticity) (Neter and Wasserman, 1974) were tested for data sets using a GLIM (Nelder, 1975) programme. A fixed effect model was employed in the ANOVA.

2.10.1 ANALYSIS OF VARIANCE: ANOVA was conducted using an SPSS (Nie *et al.* 1975) programme. A fixed effect ANOVA was used to explain the significance of variation due to major factors (e.g. cultivar, race, temperature, light intensity etc., depending on a particular experiment) and their second and higher order interactions. When the interactions, in the ANOVA table, between the major factors were significant, the interaction degrees of freedom and sum of squares were added, respectively, to the residual degrees of freedom and sum of squares, and the variance ratio for the major factors re-calculated. This adjustment permitted the discussion of the significance of the individual major factors despite the significance of the interaction terms (Chandrashekar and Heather, 1981a). The treatment means were compared employing the least significant difference (LSD) (Steel and Torrie, 1960).

2.10.2 COMPARISON OF DISEASE PROGRESS CURVES: The slope of disease progress curves (cumulative number of uredinia per leaf disk v/s days after inoculation) for different treatments was compared following

the procedure described by Sharma *et al.* (1980). Such comparisons eliminate the possibility that the differences in disease severity between any two treatments are an artifact of the time at which the disease was assessed.

2.10.3 REGRESSION ANALYSIS: Regression analysis was conducted using a GLIM (Nelder, 1975) programme. Relationship between any two factors was tested using a linear and a quadratic model. The value of coefficient of variation (R^2) produced by these models was compared and the quadratic model was adopted if it improved considerably the R^2 value.

In contrast, there are relatively few reports of the susceptibility of cultivars of *P. deltoides* to *M. larici-populina* (J. K. Sharma and Heather, 1976b; Chandrashekar and Heather, 1980) and to *M. medusae* (J. K. Sharma and Heather, 1976b), under controlled conditions in the laboratory. Such *in vitro* tests have considerable value, despite their artificiality (Steenackers, 1969). In these tests the relative resistance of cultivars to species (J. K. Sharma and Heather, 1976b) or to races (Chandrashekar and Heather, 1980) can be accurately determined and the ratings are reproducible (Chandrashekar and Heather, 1981a,c). Although cultivars are usually more susceptible under *in vitro* screening than under field conditions, the relative ranking of cultivars for resistance is essentially the same under both types of test (J. K. Sharma *et al.*, 1980). Moreover, the impact of racial variation (Chandrashekar and Heather, 1980), components of the physical (Heather and Chandrashekar, 1982) and biological (I. K. Sharma and Heather, 1981; Heather and Chandrashekar, 1983) environment on the relative resistance of cultivars can be determined readily.

In this chapter the relative resistance, expressed *in vitro*, of certain cultivars of *Populus* raised in a rust-proof glass-house to *M. medusae* is reported. The resistance, to the same collection, of cultivars of *P. deltoides*, raised in a nursery from seed collected over a range of *ca.* 14° of latitude in the U.S.A., is reported also. A field collection of urediniospores, rather than inoculum consisting of separate mono-urediniospore races (J. K. Sharma and Heather, 1976a), was employed to determine if the cultivars differed in

inherent resistance to the type of mixed race inoculum likely to occur in the field. The results presented in this chapter have been reported elsewhere (Singh and Heather, 1982a) also.

3.2 MATERIALS AND METHODS

3.2.1 UREDINIOSPORE INOCULUM: In March 1980, urediniospores of *M. medusae* were collected from twenty randomly selected cultivars (actually clones) of *P. deltoides* located in the nursery of the Botany Department, Australian National University, Canberra. These collections were bulked and referred to hereafter as a 'field collection' and stored in a refrigerator (section 2.4.1, Chapter 2). For every experiment a fresh crop of contaminant-free urediniospores was produced by inoculation with the 'field collection' of surface treated leaves of *P. X euramericana* cv. *I-488* as described previously (section 2.5.1, Chapter 2).

3.2.2 PLANTS:

Leaves from two types of plants were employed in the experiments.

3.2.2.1 PLANTS RAISED IN A RUST-FREE GLASS-HOUSE. Ten replicates of rooted cuttings of each of twenty one cultivars of *Populus* (Table 3.1) were raised in a glass-house, temperature controlled (15/25°C diurnal temperature) with a 16h (per 24h) photoperiod. Plants were maintained as described previously (section 2.2.2, Chapter 2). The development of shoots on these plants was recorded and for the

inoculation experiment, leaves of approximately the same maturity, from shoots of the same age (J. K. Sharma *et al.*, 1980) were harvested from each cultivar.

Included among these cultivars, raised in the glass-house, were the *P. deltoides 'W'* series. The latter had been produced by vegetatively propagating selected seedlings raised from seed produced by controlled hybridization of *P. deltoides* cv. *T-173* (♀) and cv. *61/58* (♂) (R. Willing, Unpublished data). At the four-leaf stage the seedlings, established in seed trays in the glass-house, had been sprinkled with the urediniospores of a field collection of leaf rust. Seedlings unaffected by this inoculation were vegetatively propagated and subsequently established as clones in the field plots of the Department of Botany, A.N.U., as the W 79/- series. Clones of the 'W' series used in Experiment 1 were selected, after 1 year of growth in the field plots, from the overall series on the basis of desirable growth rate, branching habit and apparent field resistance to leaf rusts. The other cultivars used in this experiment had not been screened previously for resistance to leaf rust.

3.2.2.2 PLANTS RAISED IN THE FIELD. Replicated plants (clones) of 42 cultivars of *P. deltoides* (Table 3.4) were available in the nursery of the Division of Forest Research, CSIRO, A.C.T. These cultivars had been propagated from selected seedlings raised from seed collected under the auspices of the U.S. Poplar Council from mother trees of *P. deltoides* growing over a latitudinal range of *ca.* 30-44°N in the U.S.A. The cuttings had been planted in the nursery in late August

1980 and during the summer of 1980-81 poplars in Canberra were almost rust-free. *M. medusae* did not appear on the cultivars in the nursery until late March/April 1981. Leaves for use in the inoculation experiment were harvested from the mid portion of the shoots (to ensure maximum and reproducible rust susceptibility (J. K. Sharma *et al.*, 1980) of these cultivars in February 1981. Some of the harvested leaves were surface treated and incubated (section 3.2.1, *ibid.*) and rust pustules did not develop.

3.2.3 INOCULATION AND INCUBATION. Inoculation followed the procedures and observed the precautions detailed elsewhere (section 2.5.2, Chapter 2). In Experiment 1, twenty disks were cut from surface treated (section 2.5.1, Chapter 2) leaves of each cultivar raised in the glass-house. These were randomized, placed in separate polythene bags, and stored briefly in a refrigerator prior to inoculation. Five leaf disks of each cultivar were selected at random, located, abaxial face uppermost, by a system of random numbers, on the base of a spore-settling tower and inoculated (section 2.5.2, Chapter 2) with 5mg of urediniospores of the 'field collection' of *M. medusae*. This procedure was repeated until twenty leaf disks of each cultivar had been inoculated. The inoculated disks were placed on plastic foam (section 2.6.2, Chapter 2) and incubated at 20±1°C; light intensity 100 μE $m^{-2}s^{-1}$ (16h photoperiod) for 14 days.

In Experiment 2, twenty leaf disks were cut (section 2.3.3, Chapter 2) from surface treated leaves harvested from each cultivar in the field and inoculated following the same procedures as in the Experiment 1.

3.2.4 DATA RECORDED. In Experiment 1, disease development on the individual disks of each cultivar was assessed on the following parameters: (i) incubation period (days) to flecking (IPF); (ii) latent period (days) prior to eruption of the first uredinium (LP1); and (iii) number of uredinia per leaf disk (ULD) after 14 days of incubation. The mean and standard error of a parameter for a cultivar was calculated from the twenty replicate disks. Infection type of each cultivar was scored on an arbitrary qualitative scale (section 2.8.1, Chapter 2).

In the second experiment, cultivars were assessed on a numerical scale (0-4, representing increasing levels of susceptibility), based on a combination of the qualitative and quantitative data. The values for the scale were: 0, immune; 1, highly resistant (necrotic flecks only, no uredinia); 2, mesothetic (necrotic flecks and occasional sporulating uredinia, some surrounded by necrotic areas); 3, susceptible (few uredinia, < 25 per leaf disk); and 4, highly susceptible (numerous uredinia, > 25 per leaf disk).

3.2.5 STATISTICAL ANALYSIS. Since, in Experiment 1, it was intended to use one-way analysis of variance to test the significance of cultivar origin with respect to disease as expressed on the leaf disks, the crude data for each of the parameters were tested for homoscedasticity and normality by means of a GLIM programme (Nelder, 1975). While the data for IPF and LP1 satisfied these pre-requisites, that for ULD did not, hence the data for the latter were transformed $[\log_e(ULD+1)]$ to fulfil these assumptions. For each parameter of

disease expression, comparison between cultivars was made using least significant difference (LSD) (Steel and Torrie, 1960). Regression analysis, employing both linear and quadratic models, was employed to assess correlations between all combinations of the parameters of disease expression.

For Experiment 2, a linear regression was fitted between numerical disease categories and corresponding latitude of the mother trees of the cultivars.

3.3 RESULTS

3.3.1 EXPERIMENT 1. Cultivars of *Populus* species, raised in the glass-house, showed a range of infection types from 1 (hypersensitive necrosis - cultivar *W 79/301*) to 4 (highly susceptible), to the 'field collection' of *M. medusae* (Table 3.1). Reaction type 4 occurred in certain cultivars of *P. deltoides* of both the *67/PC-* and *W 79/-* series, and also in the *P. alba* cv. *hickeliana*, *P. X euramericana* and the *P. nigra* cultivars. The analysis of variance demonstrated that for all the parameters employed to assess the disease severity the cultivars differed very significantly ($P = 0.001$) (Table 3.2).

Long IPF and LP1, and low ULD, all indicative of high resistance, were frequently associated in certain cultivars of the *P. deltoides 67/PC-* series, e.g., cvv. *10-3, 40-2, 123A-8* and *123A-11*. In contrast, in cultivars of the *P. deltoides 'W'* series long IPF and

Table 3.1 Mean disease intensity (3 parameters) and infection type induced in cultivars of *Populus* by a field collection of *M. medusae*.

Cultivar	Mean disease intensity			Infection
	IPF[A] (days)	LPU[A] (days)	$\log_e$ (ULD[A]+1)	type
P. alba 'hickeliana'	-[C]	8.27*a*[B]	0.94*gh*	4
P. deltoides 67/PC series				
7-1	4.75*ab*	9.43*b*	0.62h*i*	3
7-2	4.45*a*	8.32*ac*	1.04*fg*	3-4
7-4	6.20*d*	7.75*acd*	3.37b	4
10-3	8.35*g*	10.37*e*	1.18*fg*	4
40-2	8.00*g*	11.12*f*	0.94*gh*	3-4
123A-8	11.25*h*	13.50*m*	0.25*i*	3
123A-11	6.50*e*	9.09*abcekn*	0.57*i*	3-4
P. deltoides 'W' series				
79/301	6.05*de*	-[D]	-	1
79/302	7.35*f*	9.60*be*	2.06*de*	4
79/303	4.95*ab*	7.60*acdegh*	3.91*a*	4
79/304	6.05*de*	8.55*abcdi*	3.71*ab*	4
79/305	5.75*cd*	9.53*bghj*	1.36*f*	x
79/306	4.75*ab*	8.30*acdik*	2.24*d*	x
79/307	4.85*ab*	6.15*l*	3.74*ab*	4
P. deltoides T-173	5.35*bc*	8.20*acdhikno*	1.82*e*	3
P. X euramericana				
I-154	5.25*bc*	6.87*hl*	1.08*fg*	2
I-214	4.60*a*	6.05*l*	2.99*c*	4
I-488	4.90*ab*	6.35*l*	2.83*c*	4
P. nigra				
'evergreen'	5.15*b*	6.50*l*	2.42*d*	4
'italica'	5.25*bc*	6.70*l*	2.34*d*	4

A IPF - incubation period to flecking, LPU - latent period to production of first uredinium, ULD - number of uredinia per leaf disk.

B Within columns values followed by the same superscript do not differ significantly $P = 0.01$

C IPF not determinable due to hairiness

D Uredinia did not develop on this cultivar.

Table 3.2 Analysis of variance of three disease parameters, recorded on 21 cultivars of *Populus* inoculated with the 'field collection' of urediniospores of *M. medusae*.

Disease Parameter	Source of Variation	df	Sum of Squares	Mean sum of Squares	Variance Ratio
IPF	Cultivar	19	1053.087	55.426	122.56*
	Residual	380	171.848	0.452	
	Total	399	1224.936		
LP1	Cultivar	19	894.723	47.091	47.302*
	Residual	336	334.498	0.996	
	Total	355	1229.221		
ULD	Cultivar	19	506.489	26.657	141.297*
	Residual	374	70.560	0.189	
	Total	393	577.049		

* Variance ratio significant at $P = 0.001$.

LP1 were frequently not associated with low ULD, e.g., cvv. *W 79/302, W 79/304*. This contrast in behaviour is demonstrated by the low R^2 values (not significant at $P = 0.05$, in either the linear or quadratic model) between IPF and ULD, and LP1 and ULD, for the overall data (Table 3.3). However, the correlation ($R^2 = 0.73$) between IPF and LP1 is significant ($P = 0.05$).

Irrespective of the parameter employed, the constitution of the cultivar is a very significant ($P = 0.001$) determinant of the level of disease induced by the 'field collection' of *M. medusae* (Table 3.2). Among the *P. deltoides* cultivars, *W 79/301* produced chlorotic flecking only and thus was the most resistant genotype while *67/PC-123A-8* had a significantly longer IPF and LP1 than any other cultivar. On the basis of ULD, *7-2, 123A-8* and *123A-11* of the *67/PC-* series had the lowest and, *67/PC-7-4, W 79/303, W 79/304* and *W 79/307* the highest disease rating. On most parameters the cultivars of *P. X euramericana, P. nigra* and *P. alba* were susceptible to very susceptible.

3.3.2 EXPERIMENT 2. The disease category rating of the field grown cultivars, raised from seed collected in the U.S.A., varied from 0 (immune, resistant) to 4 (highly susceptible) with most cultivars rating 2/3 (Mesothetic/susceptible) (Table 3.4). Cultivars *202-1, 202-3* and *207-1*, raised from seed of mother trees located in southern Illinois, were the most resistant (rated 1, immune) while highly susceptible cultivars were derived from mother trees distributed throughout the latitudinal range. The regression analysis ($R^2 = 0.02$, *n.s.* at $P = 0.05$) indicates that disease category is independant of

Table 3.3. Variance ratio and coefficient of determination (R^2) calculated from (a) linear and (b) quadratic regression models between three parameters of disease intensity induced in cultivars of *Populus* by a field collection of *M. medusae*

Parameters Compared	Model	Variance ratio	R^2
IPF[A] v/s LPU[A]	a	46.19[B]	0.73
	b	46.40[B]	0.73
IPF v/s ULD[A]	a	0.51*ns*	0.03
	b	2.46*ns*	0.14
LPU v/s ULD	a	0.01*ns*	0.00
	b	0.04*ns*	0.00

[A] IPF - incubation period to flecking, LPU - latent period to production of first uredinium, ULD - number of uredinia per leaf disk (transformed $\log_e(ULD+1)$).

[B] Significant at $P = 0.005$, *ns* - not significant ($P = 0.05$).

Table 3.4 Disease category rating of cultivars of *Populus* raised from seed collected from mother trees at different latitudes in U.S.A., to a field collection of *M. medusae*.

Cultivar	x^A	y^B	Provenance	Latitude	Disease Category
PC/74-	*1*	*1*	Lousiana	30°21'	3
PC/74-	*2*	*1*	Lousiana	30°21'	3
PC/67-	*5*	*1*	"	30°22'	1
PC/67-	*10*	*3*	"	30°21'	2
PC/67-	*20*	*3*	"	30°16'	1
PC/67-	*24*	*2*	Mississippi	32°37'	1
PC/67-	*28*	*2*	"	32°39'	3
PC/67-	*28*	*11*	"	32°39'	1
PC/74-	*30*	*1*	Louisiana	30°30'	3
PC/74-	*39*	*2*	"	33°08'	1
PC/74-	*40*	*6*	Mississippi	33°08'	2
PC/74-	*41*	*1*	"	33°40'	2
PC/74-	*42*	*1*	"	33°40'	1
PC/74-	*42*	*3*	"	33°40'	4
PC/74-	*43*	*1*	"	33°40'	4
PC/67-	*51*	*3*	"	33°31'	1
PC/67-	*56*	*3*	Tennessee	35°47'	4
PC/67-	*60*	*31*	Tennessee	35°49'	4
PC/67-	*63*	*10*	"	35°52'	3
PC/67-	*65*	*6*	"	35°52'	2
PC/67-	*65*	*9*	"	35°52'	3
PC/67-	*65*	*10*	"	35°52'	4
PC/74-	*80*	*1*	Missouri	37°00'	3
PC/67-	*93*	*5*	Texas	30°20'	3
PC/67-	*94*	*8*	"	30°20'	3
PC/67-	*95*	*22*	"	30°20'	2
PC/67-	*109*	*2*	Illinois	40°20'	2
PC/67-	*119A-*	*2*	Illinois	37°45'	3
PC/67-	*123A-*	*8*	"	37°35'	3
PC/67-	*123A-*	*11*	"	37°35'	3
PC/67-	*146A-*	*6*	"	40°20'	4
PC/67-	*152A-*	*11*	"	40°15'	3
PC/67-	*154A-*	*1*	"	40°35'	3
PC/67-	*174*	*29*	Kansas	38°05'	3
PC/67-	*187*	*3*	Ohio	41°25'	2
PC/67-	*191*	*3*	"	41°45'	2
PC/67-	*200*	*8*	"	39°55'	4
PC/67-	*201*	*11*	Wisconsin	44°10'	4
PC/74-	*202*	*1*	Illinois	37°08'	0
PC/74-	*202*	*3*	Illinois	37°08'	0
PC/74-	*204*	*1*	"	37°43'	1
PC/74-	*207*	*1*	"	39°13'	0

PC - US Poplar Council. 67 and 74 - years of entry into Australia

x^A- open pollinated mother tree, y^B- seedling number from mother tree

latitudinal location of the mother trees of the seed from which the cultivars were raised. Plants of cultivars grown in the glass-house were more susceptible than those of the same cultivar raised in the nursery e.g. *67/PC-10-3*, infection type 4 in glass-house plants (Table 3.1), but disease category 2 in nursery plants (Table 3.4).

3.4 DISCUSSION

The results of Experiment 1 emphasize the high significance of the cultivar constitution of *P. deltoides* in determining infection type and disease level, as recorded on three parameters, induced by the 'field collection' of *M. medusae* (Table 3.1). This is in agreement with field observations on this host/pathogen system (Nagel, 1955; Dawson, 1974; Palmberg, 1977). Thus as suggested (Thielges and Adams, 1975), selection and propagation of resistant cultivars has the potential for substantial improvement in rust resistance.

The susceptibility of cultivars of *P. nigra, P. X euramericana* and *P. alba* (Table 3.1) suggests that these might not be suitable as sources of resistance in future breeding programmes.

The low, non-significant, correlations between IPF and ULD and LP1 and ULD (Table 3.2) contrast with those demonstrated for races of *M. larici-populina* and cultivars of *P. X euramericana* (Chandrashekar and Heather, 1981a). The use of a 'field collection' rather than individual races and the occurrence of 'slow rusting' cultivars, e.g. *67/PC 10-3, 7-2, 123A-8* and *123A-11*, possibly accounts, in part, for this lack of correlation. Parlevliet and van Ommeren (1975) have

demonstrated that in leaf rust of barley the correlation between latent period and rust level is much lower in slow-rusting, than in fast-rusting cultivars. The highly significant correlation between IPF and LP1 confirms previous observations with *M. larici-populina* leaf rust (J. K. Sharma *et al.*, 1980). This observation is useful since IPF can be readily and accurately determined in most cultivars at four to six days after inoculation following incubation under optimum environmental conditions.

In Experiment 2, the very poor correlation between latitude of origin of the mother tree and disease category of the cultivar, while it agrees with that of Burdon and Marshall (1980) for leaf rust in *Glycine* species, contrasts with observations recorded in the field for leaf rust in poplar (Thielges and Adams, 1975). Possibly the limited range of cultivars employed in the present experiment explains this discrepancy, however, the contrast may be more fundamental. The observations of Thielges and Adams (1975) resulted from weekly rating of replicated, individual trees of cultivars established in a single progeny test at Wooster, Ohio, i.e., the ranking of cultivars for resistance included effects due to cultivar genotype, pathogen biotypes and environment and thus was not a measure of cultivar resistance alone. While two families of *P. nigra* included in that experiment were almost completely resistant (Thielges and Adams, 1975), in Experiment 1 reported here, cultivars of *P. nigra* were very susceptible to the field isolate of *M. medusae* under the conditions of inoculation and incubation. This suggests considerable differences in racial constitution of the pathogen

and/or environment in the two experiments. The F_1 seedling progeny of hybridization between *P. deltoides* cv. *T-173* (♀) and cv. *61/58* (♂), demonstrate extreme segregation for resistance to leaf rust, e.g., *W 79/301* (immune) to *W 79/303*, *W 79/304*, and *W 79/307*) (very susceptible) (Table 3.1). Taken together these observations indicate that provenance (*sensu* latitudinal location) of the mother tree, is not a satisfactory basis for selection for resistance to *M. medusae* leaf rust, despite the observation that, in Illinois, there are significant differences in rust-resistance between certain latitudinal populations of *P. deltoides* (Jokela, 1966).

In the present experiments, cultivars showed a wide range of both qualitatively and quantitatively distinct reactions. From the mechanisms involved in the evolution of host/pathogen systems (Person, 1959), one can assume a similar related variation to be present in the pathogen population also. Experiments performed to test this assumption are reported in the following chapters 4 and 5. A further discussion of the results is given in Chapter 9.

CHAPTER 4

RECOGNITION OF PHYSIOLOGIC RACES IN *MELAMPSORA MEDUSAE* AND SENSITIVITY OF THE QUALITATIVE RACE/CULTIVAR REACTIONS TO INCUBATION TEMPERATURE AND LIGHT INTENSITY.

4.1 INTRODUCTION

It is essential to learn as much as possible about variability in fungi pathogenic to plants if we are to understand a current plant disease situation and to predict future developments (Stackman and Christensen, 1953).

The rusts are highly variable group of biotrophic fungi pathogenic to plants. Eriksson (1894) was the first to discover pathogenic specialisation on different plant species (interspecific specialisation). Barrus (1918) was probably the first to demonstrate intraspecific variation. He showed that isolates of *Colletotrichum lindemuthianum* (Sacc. & Magn.) B. C. varied in their ability to cause disease on different cultivars of *Phaseolis vulgaris* L. Subsequently, Stakman and Levine (1922) reported variability in *Puccinia graminis* Pers. f.sp. *tritici* Erikss. & Henn. and clearly established the concept of a physiologic race. Physiologic races are morphologically alike forms within a species which differ in their reactions on a set of host lines called differentials. In the last four to five decades, a huge volume of literature on specialisation and its genetic control

in pathogenic fungi has been published (Johnson and Newton, 1946; Stakman, 1947; Johnson, 1953; Day, 1960, 1974; Hooker, 1967; Watson, 1970; Hooker and Saxena, 1971; Flor, 1971; Loegering, 1978; Ellingboe, 1981).

It has become increasingly evident that environmental conditions, in particular temperature and light conditions, affect the host/pathogen reactions (Jones, 1924; Levine, 1928; Walker, 1965; Dimock, 1967; Colhoun, 1973, van der Plank, 1978). Mains and Jackson (1926) reported that the cultivar *Hussar* of wheat was generally highly resistant to some physiologic races of *Puccinia recondita* Rob. ex Desm. f.sp. *tritici* Erikss. and Henn. when inoculated in autumn or winter but only moderately or slightly resistant when inoculated in late spring. Gassner and Straib (1932a) also reported temperature sensitivity of the infection type of some wheat cultivars inoculated with urediniospores of *P. recondita*. Johnson and Newton (1937) observed that the pattern of reaction of differential cultivars of wheat to a given race of *P. graminis* f.sp. *tritici* developed at 20°C did not necessarily hold when the prevailing temperature was about 10°C higher. Several cultivars of wheat resistant to races of *Puccinia graminis* f.sp. *tritici* at 13°C night and 27°C day temperature showed little or no resistance when day and night temperature was 24°C and 27°C respectively (Newton *et al.*, 1940). Certain cultivars of wheat containing *Sr*6 genes for resistance to certain races of *P. graminis* f.sp. *tritici* were resistant at 15 but susceptible at 27°C (Watson and Luig, 1968a). Similar changes in reactions of wheat cultivars to *P. graminis* f.sp. *tritici* were

subsequently reported by Shukla (1953), Green and Johnson (1955), Hayden (1956), Forsyth (1956), Mohamed (1961), and Bromfield (1961a, b). The importance of environmental factors in the infection of wheat by *P. recondita* has also been emphasized (Hassenbrauk, 1940; Fuchs, 1960). Zimmer and Schafer (1961) and Saari and Moore (1962) stressed the role of temperature in locating new sources of resistance to *Puccinia coronata* (Cda.) Frazer and Lecl. in oats. Variable effects of light intensity on the reactions of cereal hosts to rusts have been reported (Gassner, 1927; Johnson, 1931; Hart and Zaleski, 1935; Melander, 1935, Eyal and Peterson, 1967).

Chandrashekar and Heather (1982) recently reported the differential effect of combinations of temperature and light intensity regimes of incubation on the reactions of cultivars of *Populus* species to races of *M. larici-populina*. Singh and Heather (1982c) reported a significant effect of temperature and light intensity on germination of urediniospores of *M. medusae*. They emphasized the importance of combinations of temperature and light intensity because of the significant effect of their interaction. Waterhouse and Watson (1943) reported the temperature sensitivity of reactions of certain differential cultivars of *Linum usitatissimum* L. to some races of *Melampsora lini* (Pers.) Lev.

This chapter reports; a, recognition of physiologic races in *M. medusae*; b, effects of incubation temperature on qualitative race/cultivar reactions; c, sensitivity of race/cultivar reactions to combinations of incubation temperature and light intensity. The importance of physiologic specialisation and environmental

sensitivity of race/cultivar reactions, in the ecology and epidemiology of *M. medusae* leaf rust of poplar, is discussed. A part of the results reported in this chapter has been published elsewhere (Singh and Heather, 1982b, 1983a).

4.2 MATERIALS AND METHODS

Procedures for raising mono-urediniospore isolates (section 2.4.2, Chapter 2) of *M. medusae*, cultivars (section 2.2.1, 2.2.2, 2.2.4, Chapter 2) of *Populus*, inoculation (section 2.5.2, Chapter 2) and incubation (section 2.6.2, chapter 2) of leaf disks have been described. Ten mono-urediniospore isolates of *M. medusae* were multiplied on detached leaves of *P. X euramericana* cv. *I-488* to obtain sufficient inoculum of each isolate. Hopefully, this uniform procedure avoided possible host-induced changes in the pathogen genotype during the process of multiplication. Information on the source of mono-urediniospore isolates employed is given in Appendix 3c. In the experiments reported here the inoculated leaf disks were initially incubated for 24h in dark at 20±1°C prior to their allocation to different treatments. This ensured uniform germination of urediniospores and subsequent penetration of the leaf disks by the fungus (Spiers, 1978; Singh, Unpublished data). Three experiments were conducted.

4.2.1 EXPERIMENT 1. Five replicate leaf disks of each of twenty one cultivars (Table 4.1) of *Populus* were arranged randomly on the base of a spore settling tower and inoculated separately with ten

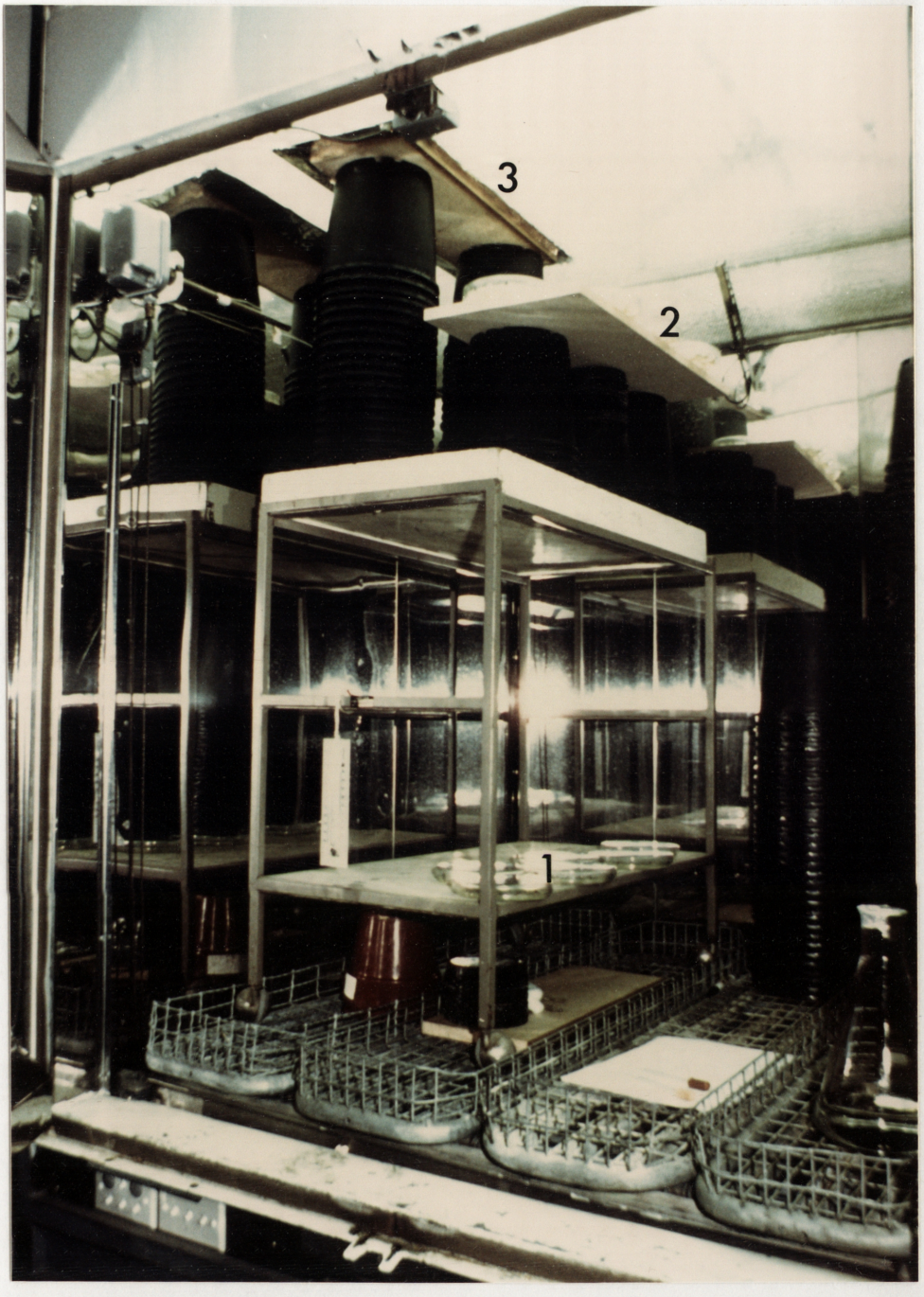

Plate 4 Three light intensity (100, 500 and 1000 μE m^{-2} s^{-1}) regimes achieved by incubating the inoculated leaf disks at different heights (1, 2 and 3 respectively) in an L. B. phytotron cabinet (Morse and Evans, 1962).

individual mono-urediniospore isolates and incubated at 20±1°C/100 μE $m^{-2}s^{-1}$.

4.2.2. EXPERIMENT 2. Fifteen replicate leaf disks of each of certain selected cultivars (Table 4.2) were inoculated with six individual mono-urediniospore isolates (distinguished, in Experiment 1, as six distinct races). Five inoculated leaf disks from each race/cultivar combination were incubated at 15, 20 and 25±1°C and a light intensity of 100 μE $m^{-2}s^{-1}$.

4.2.3 EXPERIMENT 3. Ten replicate leaf disks of each of four selected cultivars of *P. deltoides* (Table 4.3) were inoculated separately with six races of *M. medusae* and incubated at combinations of two temperature (15 and 25±1°C) and three light intensity (100, 500 and 1000 μE $m^{-2}s^{-1}$) regimes. The detailed procedures to achieve these combinations of temperature and light intensity of incubation have been described (Appendix 9) and an illustration is provided here (Plate 4.1).

4.2.4 DATA RECORDED. The race/cultivar reactions in all the experiments were recorded after 14 days total incubation (24h initial incubation included) using the qualitative scale described previously (section 2.8.1, Chapter 2).

4.3 RESULTS

4.3.1 EXPERIMENT 1. The results of the first experiment demonstrated

that the ten mono-urediniospore isolates employed could be recognised as races based on their (distinct) qualitative reactions on certain cultivars of *Populus* species (Table 4.1). Some cultivars reacted uniformly to all the isolates and *vice versa*. For example, irrespective of the mono-urediniospore isolate employed, cultivar *I-214* and *I-488* developed infection type 4. The number of race/cultivar combinations producing a particular infection type is illustrated in Figure 4.1. In the race/cultivar combinations studied, infection type 4 was most prevalent followed in order by 3, 1, 2, X, while 0 was the least common infection type.

4.3.2 EXPERIMENT 2. When incubated at 15, 20 or 25°C, the races reacted differentially with cultivars employed (Table 4.2), and in most instances the reactions of certain race/cultivar combinations were sensitive to incubation temperature. At 15 or 20°C the infection types of race/cultivar combinations were frequently higher (i.e. 3 or 4) than at 25°C but the reverse situation occurred in some instances (Table 4.2; Figure 4.2). At 15°C race 6 gave a susceptible reaction (infection type 4) on all the (differential) cultivars whilst at 25°C only necrotic flecks (infection type 1) were recorded on *P. deltoides* cvv. *W 79/301, 40-2, 123A-8* and *P. X euramericana* cv. *I-154*. At 15°C race 5 was virulent (infection type 3, 4 or X) on all the cultivars, while at 25°C, it was the least virulent of the six races (three cultivars of *P. deltoides* were immune).

From the results of the first and the second experiment, *P. X euramericana* cv. *I-488* appears to be a universal suscept, i.e., it

Table 4.1 Reactions of cultivars of *Populus* spp. to mono-urediniospore isolates of *M. medusae* at 20±1°C

Cultivar	Isolate									
	A*	B	C*	D*	E*	F	G	H*	I*	J
	Tentative assignement to races on the basis of cultivar/isolate reactions									
	1	1	2	3	4	4	4	5	6	6
P. alba 'hickeliana'	4	4	4	4	4	4	4	4	4	4
P. deltoides 67/PC										
7-1	3	3	3	3	3	3	3	3	3	3
7-2	4	4	3	3	4	4	4	4	4	4
7-4	4	4	4	4	4	4	4	4	4	4
10-3	4	4	4	4	4	4	4	4	4	4
40-2	4	4	1	4	0	0	0	1	1	1
123A-8	0	0	3	1	0	0	0	3	3	3
123A-11	*4*	*4*	*3*	*4*	*3*	*3*	*3*	*3*	*3*	*3*
P. *deltoides 'W'*										
79/301	1	1	1	2	1	1	1	1	1	1
79/302	4	4	4	4	4	4	4	4	4	4
79/303	4	4	4	4	4	4	4	4	4	4
79/304	4	4	4	4	4	4	4	4	4	4
79/305	3	3	3	3	3	3	3	3	3	3
79/306	X	X	X	X	X	X	X	X	X	X
79/307	4	4	4	4	4	4	4	4	4	4
P. deltoides T-173	3	3	3	3	3	3	3	3	3	3
P. X euramericana										
I-154	2	2	2	2	2	2	2	2	2	2
I-214	4	4	4	4	4	4	4	4	4	4
I-488	4	4	4	4	4	4	4	4	4	4
P. nigra										
'evergreen'	4	4	4	4	4	4	4	4	4	4
'italica'	4	4	4	4	4	4	4	4	4	4

* Mono-urediniospore isolates used as source of corresponding races in the subsequent experiments.

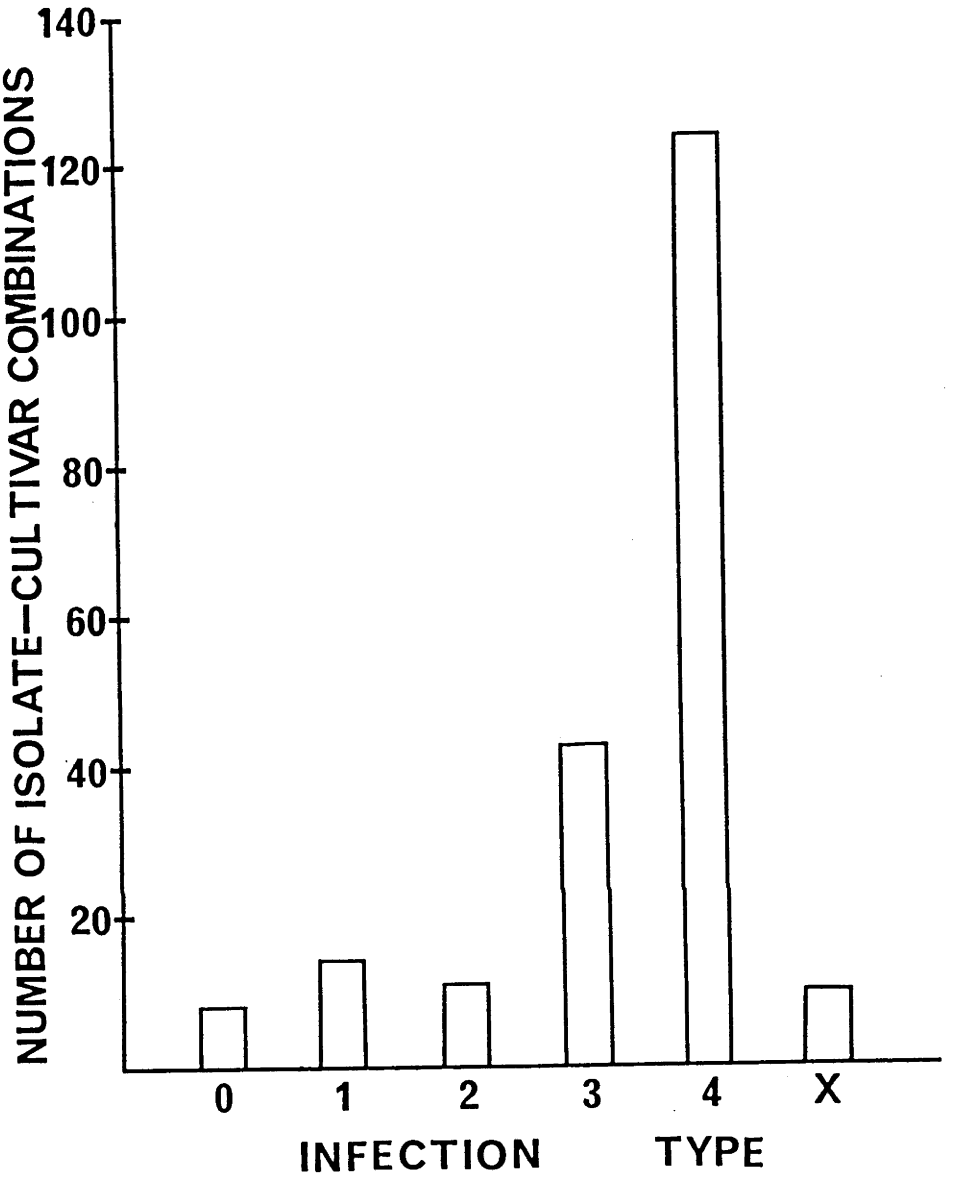

Figure 4.1 Comparison of the number of race/cultivar combinations producing different infection types (section 2.8.1, Chapter 2) when incubated at 20±1°C.

Table 4.2 Reactions* of cultivars of *Populus* spp. to races of *M. medusae* at three temperatures of incubation

Race	1			2			3			4			5			6		
Temp. of incubation	15	20	25	15	20	25	15	20	25	15	20	25	15	20	25	15	20	25
Cultivar of *Populus*																		
'7-2' +	0	4	4	0	3	4	0	3	3	4	4	4	4	4	4	4	4	3
'10-3' +	0	4	4	0	4	4	4	4	4	0	4	4	4	4	4	4	4	4
'40-2' +	4	4	3	1	1	1	4	4	3	3	0	0	4	1	1	4	1	1
'79/301' +	x	1	1	x	1	1	2	2	4	3	1	1	x	1	1	4	1	1
'79/305' +	2	2	4	3	3	3	4	4	4	4	4	4	4	3	0	4	3	4
'79/306' +	2	2	1	x	3	1	x	3	3	x	x	2	3	3	0	4	3	3
'123A-8' +	4	0	1	3	3	3	0	1	1	4	0	1	3	3	0	4	3	1
'123A-11' +	4	4	4	4	3	3	4	4	4	4	3	3	4	3	3	4	3	3
'1-488' †	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
'1-154' †	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1

+ Cultivars of *P. deltoides*; † Cultivars of *P. X euramericana*

*Host reaction scale modified from Singh and Sokhi (1980);

0 = Immune - no macroscopic symptoms; 1 = Necrotic and/or chlorotic flecks; 2 = Small uredinia surrounded by necrotic areas; 3 = Small uredinia mixed with necrotic flecks; 4 = a few to many uredinia showing copious sporulation; x = 1 + 2 + 3 + 4.

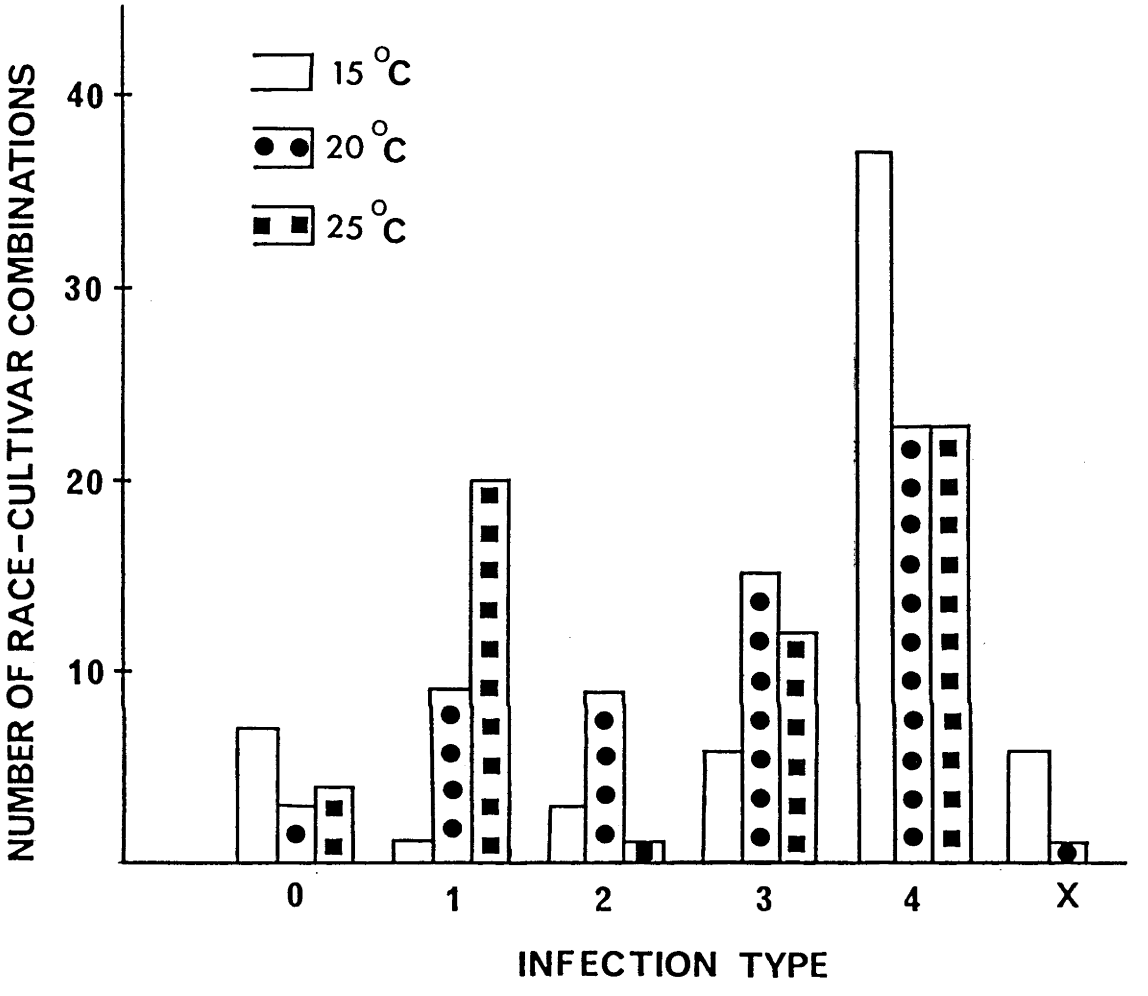

Figure 4.2 Comparison of the number of race/cultivar combinations producing different infection types (section 2.8.1, Chapter 2) when incubated at 15, 20 and 25±1°C.

gives a susceptible reaction (infection type 4) to all the races at all the temperatures of incubation, while cultivar *123A-11*, although slow-rusting (Chapter 3), was also susceptible (infection type 3 or 4) to all the races at all the temperatures. *P. X euramericana* cv. *I-154* reacted uniformly to all the races producing infection types 4, 2 and 1, respectively, when incubated at 15, 20 and 25°C.

A comparison of the cumulative race/cultivar combinations producing various infection types at three incubation temperature regimes is illustrated (Figure 4.2). Irrespective of the incubation temperature, infection type 4, as compared to 0, 1, 2, 3 or X, appeared in a greater number of race/cultivar combinations. However, for infection types other than 4, no generalised pattern occurred. At 15, 20 and 25°C respectively the relative number of race/cultivar combinations developing a particular infection type was; 0 - highest, lowest and intermediate, 1 - lowest, intermediate and highest, 2 - intermediate, highest and lowest, 3 - lowest, highest and intermediate, 4 - highest, lowest and lowest (same at 20 and 25°C), and X - highest, intermediate and lowest. This further demonstrates the differential effect of temperature of incubation on the race/cultivar reactions (infection type).

4.3.3 EXPERIMENT 3. The relative number of race/cultivar combinations developing a particular infection type at different combinations of the incubation temperature and light intensity regimes is presented (Table 4.3; Figure 4.3). When incubated at 25°C/1000 μE $m^{-2}s^{-1}$, none of the race/cultivar combinations supported uredinial production,

Table 4.3 Infection types† expressed by certain cultivars of *Populus deltoides* to infection by six races of *Melampsora medusae* when incubated at combinations of two temperature and three light intensity levels.

Race	Temp. °C	Cultivar of *P. deltoides*											
		PC-67-10-3			PC-67-40-2			PC-67-123A-8			W-79/302		
		Light Intensity ($\mu E\ m^{-2}\ s^{-1}$)											
		100	500	1000	100	500	1000	100	500	1000	100	500	1000
1	15	0	0	0	4	1	1	4	3	3	4	3	3
	25	4	3	0	3	1	1	1	1	0	1	1	0
2	15	0	0	0	1	1	1	3	3	0	4	4	4
	25	4	0	0	1	0	0	3	0	0	1	0	0
3	15	4	4	4	4	4	3	0	0	0	4	4	3
	25	4	4	0	3	3	0	1	0	0	1	0	0
4	15	0	0	0	3	1	1	4	4	4	4	4	4
	25	4	0	0	0	0	0	1	1	0	0	0	0
5	15	4	4	4	4	1	1	3	3	1	4	4	3
	25	4	3	0	1	1	0	0	0	0	1	1	0
6	15	4	4	3	4	1	1	4	0	0	4	4	3
	25	4	4	0	1	0	0	1	0	0	0	0	0

† 0 = immune - no macroscopic symptoms; 1 = necrotic and/or chlorotic flecks; 2 = small uredinia surrounded by necrotic areas; 3 = Small uredinia mixed with necrotic lesions; 4 = few to many uredinia showing copious sporulation.

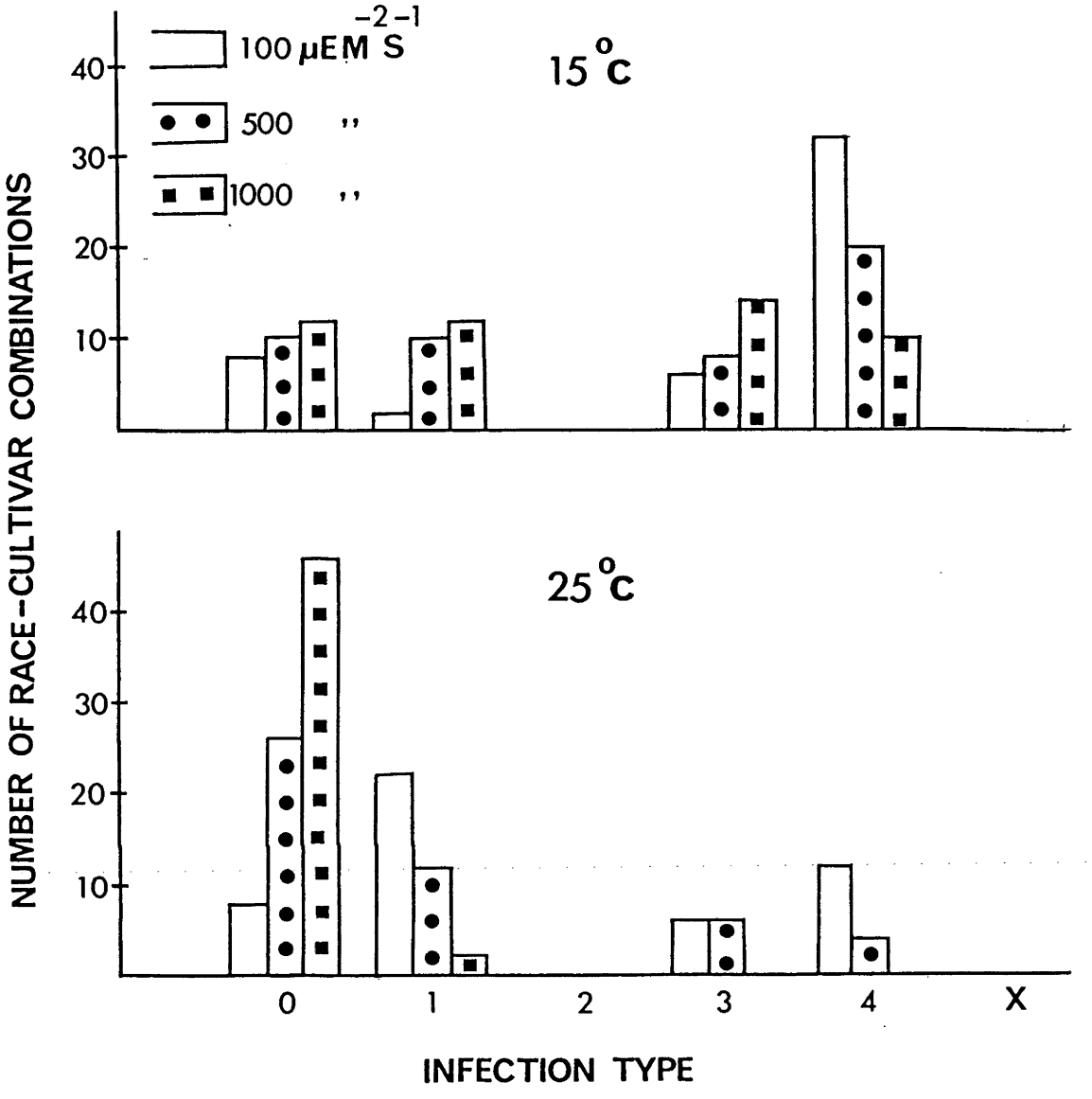

Figure 4.3 Comparison of the number of race/cultivar combinations producing different infection types (section 2.8.1, Chapter 2) when incbated at combinations of temperature (15 and 25±1°C) and light intensity (100, 500 and 1000 μE m^{-2} s^{-1}).

i.e., only infection type 0 occurred. In contrast, certain race/cultivar combinations (details not presented here), at 15°C/1000 μE $m^{-2}s^{-1}$ produced infection type 0, 1, 3 or 4. Infection type 4 (representing highest race/cultivar compatibility) developed in greatest number of race/cultivar combinations at 15°C/100 μE $m^{-2}s^{-1}$ (Figure 4.3). Thus this temperature/light intensity treatment, as compared to others, is the most congenial for disease development.

At 100 μE $m^{-2}s^{-1}$, the effect of temperature of incubation on the infection types expressed by the three cultivars of the *P. deltoides* depended on the race employed and agrees with the infection types demonstrated in Experiment 2. In contrast, in a light intensity of 100 μE $m^{-2}s^{-1}$, irrespective of the race employed for inoculation, cultivar *W 79/302* produced infection type 4 when incubated at 15°C, but infection type 0 or 1, depending on the race employed, when incubated at 25°C (Table 4.3).

In many race/cultivar combinations increasing light intensity from 100 to 1000 μE $m^{-2}s^{-1}$ resulted in lower infection type; this reduction was more pronounced at an incubation temperature of 25 than at 15°C. The infection types expressed when particular race/cultivar combinations were incubated at high temperature (25°C) and high light intensity (1000 μE $m^{-2}s^{-1}$) were as low or generally lower than those in any other light intensity/temperature treatment. In contrast, increasing either light intensity (100 to 1000 μE $m^{-2}s^{-1}$) or temperature (15 to 25°C) alone, frequently did not result in reduced infection type, e.g., for light intensity; race 3 and 5 on cultivar *PC/67-10-3* and, for temperature; race 2 on cultivar *PC/67-123A-8*.

These observations together with those of the second experiment suggest that infection type is the consequence of a differentially interactive race/cultivar/light intensity/temperature system.

4.4 DISCUSSION

The inoculated leaf disks were incubated (24h) in uniform, optimal conditions for urediniospore germination and subsequent penetration of the leaf disks by the fungus (Spiers, 1978) prior to the imposition of the temperature and/or light intensity treatments. Thus the observations reported result from the effect of the different combinations of light intensity and temperature regimes on the post-penetrative relationships of the cultivars and races.

In most instances at 15, 20 or 25°C, any two races of *M. medusae* could be distinguished by the differential reactions of a particular pair of cultivars of *P. deltoides* but not of those of *P. X euramericana* (Table 4.2). The recognition of races in *M. medusae* confirms the observations of Sharma and Heather (1976a).

In certain instances, two or three separate mono-urediniospore isolates produced identical infection types on the cultivars employed (Table 4.1). Thus on the basis of these reactions, these isolates could be considered as a single race. However, it might be possible to distinguish such isolates if some additional differential cultivars were included. Thus the spores of these isolates were not pooled and in subsequent experiments mono-urediniospore isolates; A, C, D, E, H, and I, representing race 1 to 6, only were used.

Similarly, certain cultivars reacted uniformly to all the isolates and hence these could not be used as differentials for the recognition of the races identified in the present experiments.

The generally higher resistance (lower infection type) of cultivars of *P. deltoides* to races of *M. medusae* when incubated at 25 rather than 15°C agrees with the observations of Toole (1967) and Spiers (1978). Similarly, cultivars of *P. X euramericana* and *P. deltoides* were more resistant to races of *M. larici-populina* when incubated at a high (25°C) rather than a lower (12 or 20°C) temperature (Chandrashekar and Heather, 1981a).

Resistance of certain cultivars of *P. X euramericana* to *M. larici-populina* was always higher at 25 than at 20 or 12°C (Chandrashekar and Heather, 1981a). While in the present study the pattern of resistance in many cultivars to particular races follows the same trend, in other instances this pattern is either not pronounced or is even reversed. This apparent behavioural difference between these two species of the pathogen may be purely circumstantial and result from the small number of selected races and cultivars employed in the experiments. However, this possible contrast in behaviour is worthy of a further investigation screening large number of cultivars as it may be important in the seasonal occurrence of epidemics (Singh and Heather, 1982b) of leaf rust of poplar caused by the two *Melampsora* species in Canberra.

Resistance of the cultivars was generally higher (*sensu* lower infection type) when the cultivar/race combinations were incubated in a light intensity of 1000 rather than at 100 or 500 μE $m^{-2}s^{-1}$. In

contrast, Spiers (1978) observed that light (intensity and quality unspecified) did not affect the infection of leaf disks of *P. X euramericana* cv. *I-455* by a field isolate of *M. medusae*. However high (1000), when compared with low (100 μE $m^{-2}s^{-1}$), light intensity increased the resistance (basis ULD) of compatible cultivars of *P. X euramericana* to races of *M. larici-populina* (Chandrashekar and Heather, 1981b). Reports of the effects of light intensity on the compatibility of cereal hosts to rusts are conflicting with increasing light intensity causing reduced (Gassner, 1927, Johnson, 1931), increased (Hart and Zaleski, 1935) and having no effect (Melander, 1935) on host/pathogen compatibility. Further, Melander (1935) has observed that low, when compared with high, light intensity may decrease uredinial and urediniospore size in *Puccinia graminis* f.sp. *tritici*.

Increased resistance (lower infection type) in the cultivars at higher light intensities was generally more pronounced when the race/cultivar combinations were incubated at 25 rather than 15°C, i.e., there is some evidence for a light intensity/temperature interaction in determining the level of qualitative resistance expressed by the cultivar. This interdependance resembles the differential quantitative interaction between light intensity and temperature in the disease level induced in cultivars of *P. X euramericana* by races of *M. larici-populina* (Chandrashekar and Heather, 1982).

The differential sensitivity of the race/cultivar interactions to temperature and light intensity of incubation suggests a degree of

ecological specialisation among the races of *M. medusae*. The temperature sensitivity of race-specific reactions of cultivars of poplar demonstrated here and elsewhere (Chandrashekar and Heather, 1981a,b, 1982) parallels that occurring in stripe rust and stem rust of wheat (Green and Johnson, 1955; Fuchs, 1960). Such sensitivity complicates the identification of races (Johnson *et al.*, 1967).

Independent of the races employed, resistance in the cultivars was at a maximum (infection type at a minimum) when the leaf disks were incubated at 25°C in a light intensity of 1000 μE $m^{-2}s^{-1}$. High temperature (25°C) and high light intensity 500 μE $m^{-2}s^{-1}$ are inhibitory also to the germination of urediniospores of *M. medusae* (Singh and Heather, 1982c). Thus, the effects of increasing temperature and light intensity on pre- and post-penetration phenomena with *M. medusae* are likely to reinforce one another in reducing the level of rust induced in the cultivars of poplar.

It is tempting to assume that the increased resistance of cultivars with increasing light intensity results only from the effect of light intensity on host physiology. However, increased light intensity also depresses the germination of urediniospores of *M. medusae* (Singh and Heather, 1982c), i.e., a direct effect of light on the physiology of the pathogen. Thus light intensity possibly affects directly the colonization of host tissue by the races of the pathogen even in the post-penetration phase.

The qualitative race-specific reactions of cultivars of poplar to races of *M. medusae* suggest a potential for host selection pressure on the racial composition of the pathogen population

(Heather *et al.*, 1980a). Homeostasis and stabilising selection (van der Plank, 1968) in the pathogen have been suggested as forces oppositing this pressure and thus maintaining variability in such populations. In the field, poplar cultivars will be subjected to considerable diurnal and seasonal environmental fluctuations and the sensitivity to temperature of the reaction types of race/cultivar combinations should tend also to maintain variability in the pathogen population. A further discussion of the results reported here is given in Chapter 9.

CHAPTER 5

TEMPERATURE-LIGHT EFFECTS ON QUANTITATIVE REACTIONS OF POPLAR CULTIVARS TO RACES OF *MELAMPSORA MEDUSAE* LEAF RUST.

5.1 INTRODUCTION

The effects of temperature and light intensity of incubation on the qualitative interactions of races of species of the *Uredinales* with cultivars of their hosts have been reviewed, in part, in Chapter 4. Recently, temperature (12, 20 or 25°C) (Chandrashekar and Heather, 1981a) and light intensity (100, 250 or 1000 μE $m^{-2}s^{-1}$) (Chandrashekar and Heather, 1981b) of incubation, applied separately or in concert (15 or 24°C and 50 or 200 μE $m^{-2}s^{-1}$) (Chandrashekar and Heather, 1982), affected differentially the quantitative interactions of races of *M. larici-populina* and cultivars of *Populus* species.

The qualitative reactions of six races of *M. medusae* with differential cultivars of *P. deltoides* were sensitive to the temperature (Table 4.2, Chapter 4), and light intensity (Table 4.3, Chapter 4), of incubation. Resistance in the cultivars was maximum at high temperature (25°C) and high light intensity (1000 μE $m^{-2}s^{-1}$). Despite previous observations suggesting the contrary (Spiers, 1978), the germination of urediniospores of *M. medusae* is inhibited by increasing temperature (range 15 to 25°C) and increasing light

intensity (range 0-500 μE $m^{-2}s^{-1}$) and further the temperature and light intensity of incubation interact differentially in determining the germination of urediniospores (Appendix 9).

This chapter reports a factorial experiment in which the effects of concomitant variation in temperature (2 levels) and light intensity (3 levels) of incubation, on the quantitative reactions (as assessed using 3 parameters) of leaf disks, cut from four compatible cultivars of *Populus* spp., to six races of *M. medusae* were studied. The temperature and light intensity regimes employed are characteristic of those occurring on the abaxial face of leaves during the active growing season of poplar in southeastern Australia.

5.2 MATERIALS AND METHODS

5.2.1 RAISING OF CULTIVARS OF *POPULUS* AND RACES OF *M. MEDUSAE*: Replicate, clonal cuttings of *P. X euramericana* cvv. *I-214 and I-488*, and *P. deltoides* cvv. *W 79/304 and W 79/307*, were raised under controlled conditions (temperature 20/28°C - dark/light - 16h photoperiod, natural light supplemented with artificial light) in a phytotron cabinet (Chandrashekar and Heather, 1981c). Six mono-urediniospore races of *M. medusae* (Table 4.1, Chapter 4) were multiplied in detached leaf culture on *P. X euramericana* cv. *I-488* as described elsewhere (section 2.5.1, Chapter 2).

5.2.2 INOCULATION AND INCUBATION: The general procedures for selection (section 2.3.1, Chapter 2), surface treatment of leaves

(section 2.3.2, Chapter 2), cutting of leaf disks (section 2.3.3, Chapter 2) and inoculation (section 2.5.2, Chapter 2) of these with urediniospores of the separate races followed those detailed previously. Inoculated leaf disks were placed on plastic foam, soaked in 10 p.p.m. (w/v basis) gibberellic acid in Petri plates (section 2.6.2, Chapter 2) and incubated for 24h at 20±1°C in the dark. This pre-treatment permitted uniform germination of the urediniospores and subsequent penetration of the leaf disks by the races of the fungus (Spiers, 1978, Singh, Unpublished data). Subsequently, ten replicate leaf disks of each race/cultivar combination were selected randomly and allocated for incubation at each combination of temperature (15 and 25°C) and light intensity (100, 500 and 1000 μE $m^{-2}s^{-1}$). The detailed procedures to achieve these regimes of temperature and light intensity of incubation have been described elsewhere (section 4.2.3, Chapter 4).

5.2.3 DATA RECORDED: Following incubation at the various temperature/light intensity combinations, disease development on the leaf disks was assessed on three parameters: IPF - incubation period (days) to flecking; LP1 - latent period (days) to eruption of the first uredinium; and ULD - number of uredinia per leaf disk after 14 days of incubation.

5.2.4 STATISTICAL ANALYSIS: The data were tested for homoscedasticity and normality, (Neter and Wasserman, 1974) using a GLIM programme (Nelder, 1975) and were subjected to analysis of variance using the sub-program ANOVA of SPSS (Nie *et al*. 1975). The application of these

techniques has been illustrated elsewhere (Chandrashekar, 1981). For each parameter of disease, the data for each temperature/light intensity combination were averaged across cultivars and across races. The quadratic model, $Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + E_i$, was used to compute the co-efficients of determination (R^2) (Neter and Wasserman, 1974), between IPF, LP1 and ULD for the overall data by employing the statistical computer language GLIM (Nelder, 1975).

5.2.5 DISEASE PROGRESS CURVES: (Number of uredinia per leaf disk v/s total days after incubation) for cultivars of poplar (summed across races) and for races (summed across cultivars) were prepared for different individual combinations of temperature and light intensity regimes. Similar disease progress curves, based on overall data, for individual cultivars (summed across races, temperature and light intensity) and for individual races (summed across cultivars, temperature and light intensity) were plotted.

5.3 RESULTS

Visual symptoms of disease did not develop in any of the race/cultivar combinations incubated at 25°C in a light intensity of 1000 μE $m^{-2}s^{-1}$ (Table 5.1 and 5.2, Figure 5.1 and 5.2) or in certain race/cultivar complexes (full details not included here), when incubated at particular conditions, e.g., race 3, 4, 5 or 6/*P. deltoides* cv. *79/307* when incubated at 25°C and 500 μE $m^{-2}s^{-1}$. Necrotic flecks, which were not specific to particular race/cultivar

Table 5.1 Disease severity, as measured by three parameters[a], induced in four cultivars of poplar by six races of *M. medusae* when incubated at two temperatures and three light intensities

Cultivar	Light intensity ($\mu E\ m^{-2}\ s^{-1}$)																	
	100						500						1000					
	Temperature (°C)						Temperature (°C)						Temperature (°C)					
	25			15			25			15			25			15		
	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD
I-214[c]	4.05[b]	6.10	4.75	6.07	8.43	8.38	4.63	8.10	4.07	5.83	8.68	6.20	-	-	0.00	5.75	8.43	6.97
I-488[c]	4.02	6.20	4.02	5.97	9.05	6.95	4.55	8.42	3.07	5.47	8.43	8.00	-	-	0.00	5.55	8.15	5.82
79/304[d]	4.00	6.03	27.98	5.23	8.95	36.19	4.23	7.88	17.34	5.18	8.75	32.39	-	-	0.00	5.83	8.13	35.33
79/307[d]	4.00	6.02	18.93	5.47	8.68	39.25	4.37	8.23	0.12	5.13	8.12	38.12	-	-	0.00	5.05	7.89	25.72

[a] IPF - incubation period to flecking (days), LP1 - latent period to production of first uredinium (days), ULD - uredinia per leaf disk (1.76cm^2) after 14 days of incubation in the temperature/light intensity regimes.

[b] Each value in the body of the table is the mean of ten observations (leaf disks)

[c] Cultivars of *P. X euramericana*

[d] Cultivars of *P. deltoides*. Local necrosis developed around some uredinial pustules on these cultivars after 10 days of incubation

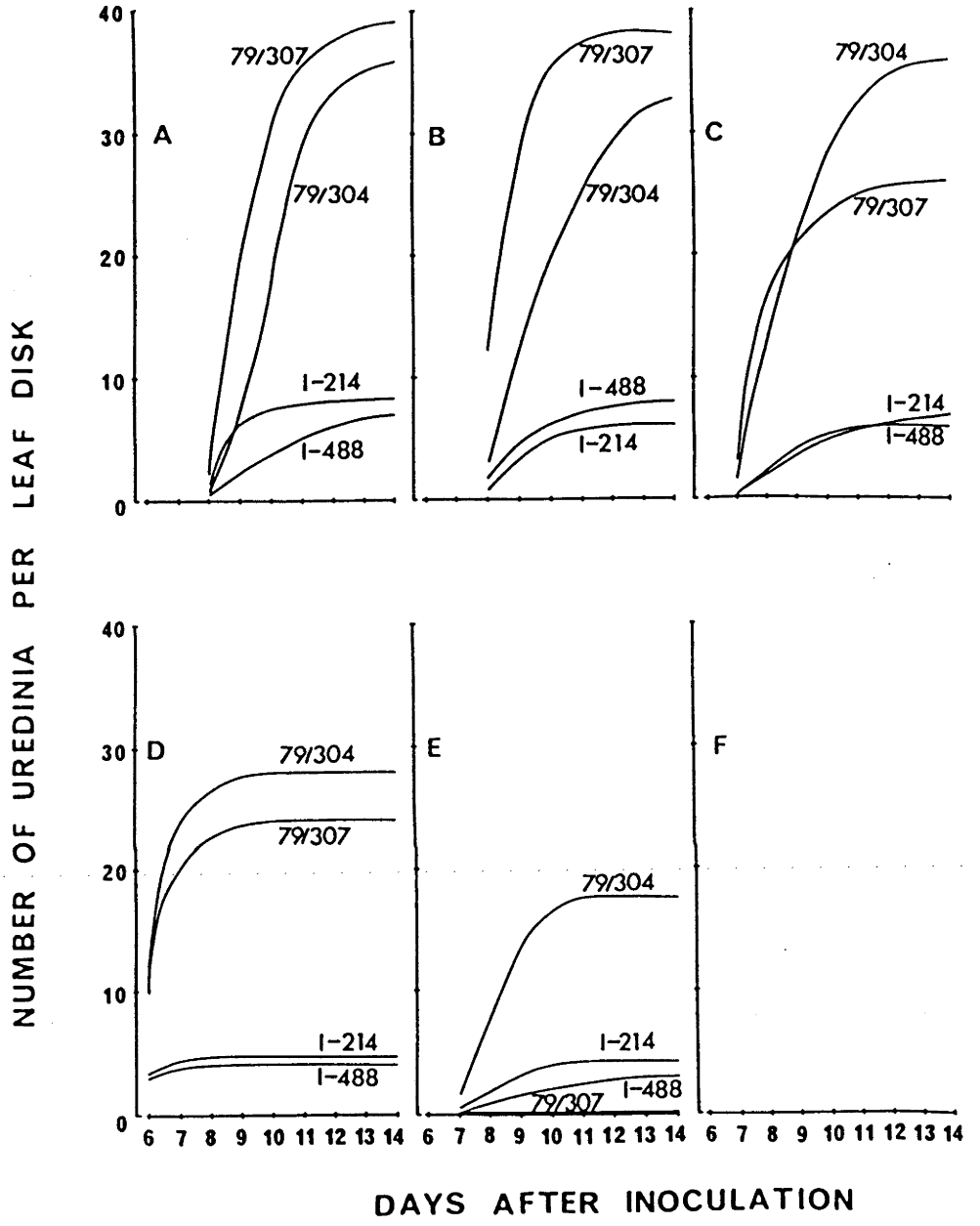

Figure 5.1 Disease progress curves (ULD) for four cultivars of *Populus* species (summed across six races of *M. medusae*) incubated at various temperature and light intensity combinations (A, 15/100; B, 15/500; C, 15/1000; D, 25/100 E, 25/500 and F, 25°C/100 μE m^{-2} s^{-1}).

Table 5.2 Disease severity, as measured by three parameters,[a] induced by six races of *Melampsora medusae* on four cultivars of poplar when incubated at two temperatures and three light intensities

Race	Light intensity ($\mu E\ m^{-2}\ s^{-1}$)																	
	100						500						1000					
	Temperature (°C)						Temperature (°C)						Temperature (°C)					
	25			15			25			15			25			15		
	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD	IPF	LP1	ULD
1	4.08[b]	6.15	14.88	5.58	8.15	25.15	4.15	7.90	7.40	5.50	8.40	21.28	-	-	0.00	5.50	7.68	19.33
2	4.00	6.08	13.30	5.50	8.98	14.65	4.00	8.00	10.88	5.50	8.65	15.03	-	-	0.00	5.50	8.31	12.40
3	4.00	6.10	14.40	6.00	8.50	16.28	4.68	8.18	3.78	5.60	8.03	18.43	-	-	0.00	5.73	8.33	10.11
4	4.13	6.13	12.25	5.68	8.83	16.68	4.50	8.17	5.82	5.00	8.43	20.70	-	-	0.00	5.00	7.71	21.18
5	4.00	6.03	11.33	5.50	8.90	39.30	4.13	7.90	5.69	5.30	8.55	30.00	-	-	0.00	5.25	7.85	32.35
6	4.00	6.05	17.38	5.80	9.33	24.11	5.13	8.68	3.55	5.53	8.93	22.66	-	-	0.00	5.30	9.03	15.39

a IPF - incubation period to flecking (days), LP1 - latent period to production of first uredinium (days), ULD - uredinia per leaf disk (1.76 cm^2) after 14 days of incubation in the temperature/light intensity regimes.

b Each value in the body of the table is the mean of ten observations (leaf disks).

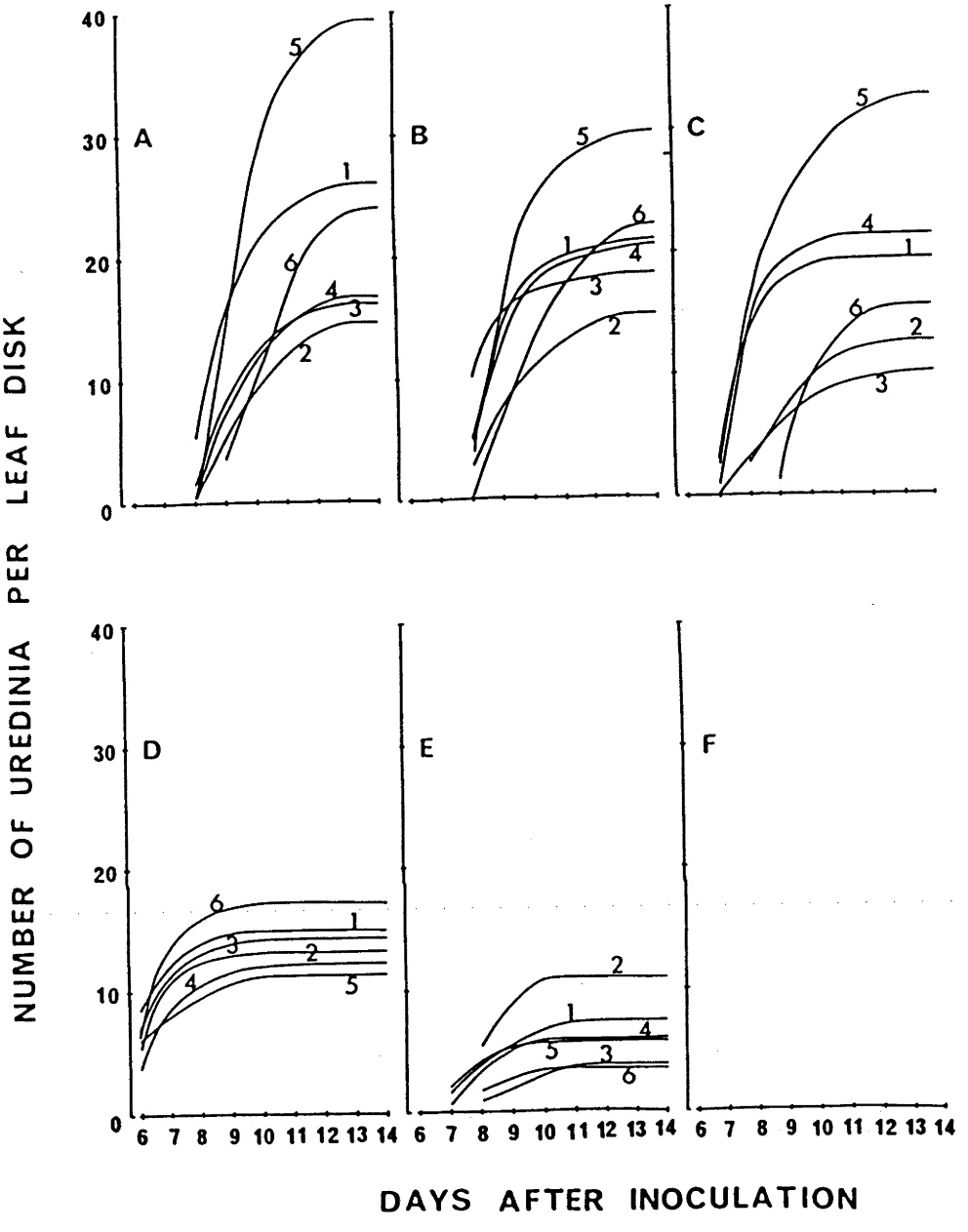

Figure 5.2 Disease progress curves (ULD) for six races (1-6) of *M. medusae* (summed across four cultivars of poplar) when incubated at different combinations of temperature (15 and 25±1°C) and light intensity (100, 500 and 1000 μE m^{-2} s^{-1}), producing different infection types (section 2.8.1,Chapter 2)

combinations, developed in some of these disks when they were subsequently transferred for incubation for 14 days at 15°C/100 μE $m^{-2}s^{-1}$, the most favourable conditions for the development of *M. medusae* leaf rust. Disks from those treatments in which disease symptoms did not develop after the original 14 days of incubation were omitted from the analysis of IPF and LP1, while a zero (0) value was inserted for these in the analysis of variance for ULD.

For the three parameters of disease the major factors (race, cultivar, temperature and light intensity), and their second, and third order interactions for ULD, were significant (P = 0.001) determinants of disease intensity (Table 5.3). Since a fixed effect model was employed in the ANOVA it is possible, following adjustment of the value of the residual variance (section 2.10.1, Chapter 2), to discuss the significance of the individual major components despite the significance of the interaction terms. Following such a re-calculation the variance ratios of the major factors are still significant (P = 0.01).

In all those race/cultivar combinations in which symptoms of disease developed after 14 days of incubation, and independant of the light intensity employed, IPF and LP1 were always shorter in disks incubated at 25 rather than 15°C (Table 5.1 and 5.2, Figure 5.1 and 5.2). This accounts for the large value of the variance due to temperature in the ANOVA for these parameters (Table 5.3). However, IPF and LP1 increased with increasing light intensity (range 100-500 μE $m^{-2}s^{-1}$) in the disks incubated at 25°C, but usually decreased, or remained stable, with increasing light intensity in those leaf disks

Table 5.3 Analysis of variance of three disease parameters recorded in four cultivars of *Populus* spp. separately interacting with six races of *Melampsora medusae* at combinations of two temperature, and three light intensity, levels.

Source of Variation	Variance ratio[b]		
	IPF	LP1	ULD
Race	280.75	82.58	23.36
Cultivar	108.97	14.20	358.21
Temperature	2353.97	1246.63	653.31
Light	15.44	103.03	95.00
Race X cultivar	-[a]	-	6.68
Race X Temp.	-	-	31.29
Race X Light	-	-	3.08
Cultivar X Temp.	-	-	114.43
Cultivar X Light	-	-	16.02
Temp. X Light	-	-	27.23
Race X Cultivar X Temp.	-	-	5.28
Race X Cultivar X Light	-	-	2.48
Cultivar X Temp. X Light	-	-	20.22
Race X Cultivar X Temp. X Light	-	-	1.19NS

Residual degrees of freedom for IPF, LP1 and ULD were 1188, 1077 and 1287 respectively.

[a] Interaction terms for IPF and LP1 were not calculated due to missing cells, i.e., race/cultivar/temperature/light intensity combinations in which symptoms did not develop after 14 days of incubation.

[b] All unmarked variance ratios are significant at $P = 0.001$.

NS Not significant at $P = 0.05$.

incubated at 15°C (Table 5.1 and 5.2). This indicates an interaction between light intensity and temperature of incubation in determining these parameters of disease. Due to missing cells in the data (combinations in which uredinia did not develop), the statistical significance of this was not determined.

Short IPF and/or LP1 are indicative of high susceptibility (van der Plank, 1968) hence, on these criteria, resistance in all race/cultivar complexes is as low, or lower, when these are incubated at 25°C/100 μE $m^{-2}s^{-1}$ rather than at any other combination of temperature and light intensity.

The temperature of incubation is also a major determinant of variation in ULD. However, this possibly results, at least in part, from inclusion in the analysis of zero values for ULD in those race/cultivar complexes in which symptoms of disease did not develop after 14 days of incubation. Despite this reservation, irrespective of whether the data for ULD are averaged across races or across cultivars, and independant of the light intensity employed, mean ULD is always higher in disks incubated at 15 rather than 25°C (Table 5.1 and 5.2). There are departures from this general pattern when individual race/cultivar complexes (detailed data not presented here) are examined and these account for the significant (P = 0.001) two-way interactions of combinations of light, temperature, cultivar and race in the ANOVA (Table 5.3). Irrespective of the cultivar or race employed, ULD in those race/cultivar combinations incubated at 25°C decreases with increasing light intensity (range 100-500 μE $m^{-2}s^{-1}$). The effect of increased light intensity (100 - 500 - 1000 μE $m^{-2}s^{-1}$),

on the race/cultivar complexes incubated at 15°C, is inconsistent and depends on the race and cultivar employed (Table 5.1 and 5.2; Figure 5.1 and 5.2). These inconsistencies, combined with the contrast in the effect of increasing light intensity on ULD in those disks incubated at 25 rather than 15°C, account for the significant two-, three- and four-way interactions of the factors in the ANOVA for ULD (Table 5.3).

As might be expected from the foregoing results, while the correlation between IPF and LP1 (R^2 = 0.61 *Sig.* P = 0.05) is reasonable, those between IPF and ULD (R^2 =0.18 *n.s.* P = 0.05) and LP1 and ULD (R^2 = 0.01 *n.s.* P = 0.05) are very low.

The cultivar is a very important determinant of ULD (Table 5.3). With one exception (cultivar *79/307* incubated at 25°C/500 μE $m^{-2}s^{-1}$), irrespective of the race, temperature and light intensity of incubation, the ULD on the cultivars of *P. deltoides* is always higher (more susceptible) than that on cultivars of *P. X euramericana* (Figure 5.1 and 5.3). However, relative ranking for susceptibility of the individual cultivars within these two taxonomic groups depends on the light intensity and temperature of incubation, e.g., ULD in cultivar *I-488* is lower than that in cultivar *I-214* when incubated in a light intensity of 100 μE $m^{-2}s^{-1}$ at 15 or 25°C but this ranking is reversed when the cultivars are incubated at 15°C in a light intensity of 500 or 1000 μE $m^{-2}s^{-1}$ (Table 5.1, Figure 5.1).

The relative resistance of poplar cultivars, as assessed according to overall progress of disease mono-cycle, is dependant on

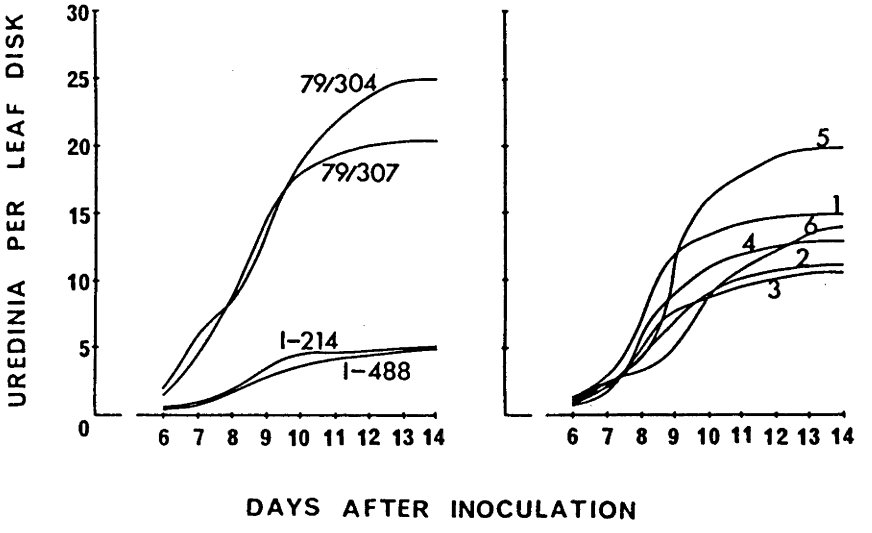

Figure 5.3 Cumulative disease progress curves (ULD) for cultivars of poplar (summed across six races, two temperature and three light intensity regimes) and for races of *M. medusae* (summed across four cultivars, two temperature and three light intensity treatments).

the combinations of incubation temperature and light intensity (Figure 5.1). For example *P. deltoides* cv. *W 79/307*, as compared to other cultivars, is the most susceptible cultivar when incubated at 15°C/100 or 500 μE $m^{-2}s^{-1}$, ranks second (after cultivar *W 79/304)* at 15°C/1000 μE $m^{-2}s^{-1}$ and 25°C/100 μE $m^{-2}s^{-1}$, but least susceptible at 25°C/500 μE $m^{-2}s^{-1}$. In most instances the relative magnitude and ranking of cultivars for resistance varies also with the time of disease assessment. For example when incubated at 15°C/100 μE $m^{-2}s^{-1}$ cultivar *W 79/307* is uniformly more susceptible than cultivar *W 79/304* at most of the disease assessment times (i.e. the disease progress curves for these cultivars are almost parallel), however, when incubated at 25°C/500 μE $m^{-2}s^{-1}$, it is always more susceptible but to a varying magnitude (i.e. the disease progress curves are not parallel) while at 15°C/1000 μE $m^{-2}s^{-1}$ it is more susceptible than cultivar *W 79/304* in the first half of disease mono-cycle but less susceptible in the second half (i.e. the disease progress curves for these two cultivars cross over) (Figure 5.1).

The race employed is a lesser, but a significant, determinant of variation in ULD (Table 5.3). When the race/cultivar complexes are incubated at 15°C, irrespective of the light intensity, race 5 is the most aggressive (highest ULD) race. However, when the complexes are incubated at 25°C, depending on the light intensity, race 6 (at 100 μE $m^{-2}s^{-1}$) or race 2 (at 500 μE $m^{-2}s^{-1}$) is the most aggressive race, while race 5 is the least aggressive at 100 μE $m^{-2}s^{-1}$ (Table 5.2, Figure 5.2). Within a mono-cycle the time of disease assessment is an important factor in determining the relative ranking of races for aggressiveness (basis ULD). For example at 15°C/100 μE $m^{-2}s^{-1}$, race 6

is the least aggressive of all the races at 9 days of incubation, more aggressive than race 2 at 10 days of incubation, and also more aggressive than races 2, 3 and 4 at 11-14 days of incubation period (Figure 5.2).

Through most of the disease mono-cycle cultivars of *P. deltoides* were generally more susceptible than those of *P. X euramericana* (Figure 5.1 and 5.3, details on reactions of cultivars to individual races not presented here). The obvious exception was *P. deltoides* cv. *W 79/307* which, as compared to other cultivars, when incubated at 25°C/500 μE $m^{-2}s^{-1}$ was the least susceptible (Figure 5.1). However, this exception is masked when the data for cultivars are pooled across all the race/temperature/light intensity combinations (Figure 5.3). Similarly, race 5, based on overall data (Figure 5.3) is the most aggressive race (at least highly so in the second half of the uredinial production period) while at 25°C/100 and 25°C/500 μE $m^{-2}s^{-1}$ this, as compared with others, was the least or moderately aggressive race (Figure 5.2). Thus, these examples demonstrate clearly that conclusions drawn from the overall data, in certain cases, fail to represent exceptions to the generalised statements (based on overall data). Such exceptions, though are relatively less important from cumulative disease progress view point but these could be very important in studies on fundamental relationships between the host and the pathogen, e.g., selection of differential host cultivars and incubation conditions for the identification of races. These exceptions further explain the poor correlation, based on overall mean data, between different disease parameters.

5.4 DISCUSSION

Prior to allocation to particular light intensity/temperature treatments the leaf disks, inoculated with the urediniospores of the races, were incubated (24h) at conditions which are optimal for germination of the spores and penetration of the leaf disks by the fungus (Spiers, 1978). Thus, the results report the effects of light intensity/temperature of incubation treatments on the post-penetrative phase of host/pathogen relationship. In addition, although the levels of temperature and light intensity employed are typical of those occurring in plantations of poplar in the field in Canberra, the form of their application, e.g., constant levels throughout the 14 days of incubation, is very artificial. Hence extrapolation from the present results to the epidemiology of leaf rust in the field situation should be made with caution.

The results demonstrate significant differences in the aggressiveness of races, assessed on various parameters of disease, and hence suggest a possibility of employing quantitative reactions as a basis of race identification. This agrees with the results of Chandrashekar and Heather (1980) which suggested the possibility of identifying races employing quantitative reactions similar to those reported here. The differentiation of races based on their quantitative reactions, as compared to qualitative reactions (Chapter 4) is considered to be much more informative (Johnson and Taylor, 1976). However, the results demonstrate a significant race X cultivar X temperature X light intensity interaction in determining the disease severity; this indicates the necessity for careful control of

the incubation environment to achieve reproducible results in such studies.

The race-specific relationship, i.e., significant race/cultivar interaction in the ANOVA (Table 5.3) of *M. medusae* and *Populus* species, and the independant effects of temperature and light intensity on disease level (basis ULD), induced in cultivars of *P. deltoides* and *P. X euramericana* by races of *M. medusae*, confirm results reported and discussed elsewhere (Chapter 4), and parallel those observed for the *M. larici-populina* race/*P. X euramericana* cultivar system (Chandrashekar and Heather, 1981a,b, 1982). These separate effects will not be discussed further.

The compatibility (basis ULD) of race/cultivar combinations was highest when these were incubated at low temperature (15°C) and low light intensity (100 μE $m^{-2}s^{-1}$) regime. Uredinia did not develop when race/cultivar combinations were incubated at 25°C/1000 μE $m^{-2}s^{-1}$ light intensity combination. This agrees with the results on effects of similar temperature and light intensity combinations on the qualitative reactions of cultivars of *Populus* species to races of *M. medusae* (Table 4.2 and 4.3, Chapter 4). Chandrashekar and Heather (1982) reported a similar interaction of temperature and light intensity of incubation on the development of *M. larici-populina* leaf rust on compatible cultivars of *Populus* species.

When IPF, LP1 and ULD are averaged across cultivars and races and compared between different couplings of temperature and light intensity, in those combinations in which uredinia were present after 14 days of incubation, mean IPF (4 days) and mean LP1 (6.1 days) are

shortest in race/cultivar complexes incubated at 25°C/100 μE $m^{-2}s^{-1}$ while ULD is the highest in those complexes incubated at 15°C/100 μE $m^{-2}s^{-1}$. This example is characteristic of the overall data, hence the correlations between IPF/LP1 or ULD are low and non-significant (details not presented here). This agrees with the observations on the effect of different couplings of light intensity and temperature on IPF and ULD in the *M. larici-populina/P. X euramericana* complex (Chandrashekar and Heather, 1982). Both these sets of observations contrast with the general inverse correlation between IPF and ULD when the separate effects of light (Chandrashekar and Heather, 1981b) and temperature (Chandrashekar and Heather, 1981a), on the reactions of the *M. larici-populina/P. X euramericana* systems, were studied. This suggests that the interaction of light and temperature affects differentially the rate of fleck production and the number of sporulating pustules that develop. The lack of generalised negative correlation between IPF and ULD for the poplar leaf rusts caused by both *M. medusae* and *M. larici-populina* contrasts with the cereal rusts where reduction in latent period, of which IPF is a portion, with increasing temperature from *ca.* 10 to 25°C (Eversmeyer *et al.*, 1980; Teng and Close, 1978) is inversely correlated with uredinial number per unit area. Due to the absence of such a generalised correlation in leaf rust of poplar, the relative ranking of cultivars for resistance and of races for aggressiveness, in this system will depend, to a degree, on the disease parameter employed.

Disease severity (basis ULD), and hence the apparent relative resistance of cultivars and expressed relative aggressiveness of

races, is dependant on the combination of incubation temperature and light intensity. When incubated at 15°C/100 or 500 μE $m^{-2}s^{-1}$ race 5 is the most, and race 2 the least aggressive race. In contrast, when incubated at 25°C/100 μE $m^{-2}s^{-1}$, race 5 is the least aggressive race and at 25°C/500 μE $m^{-2}s^{-1}$ race 2 is the most aggressive race (Table 5.2). Similarly, *P. deltoides* cv. *W 79/307* is the most susceptible cultivar when incubated at 15°C/100 or 500 μE $m^{-2}s^{-1}$ but it is the most resistant cultivar when incubated at 25°C/500 μE $m^{-2}s^{-1}$. For these couplings of temperature and light intensity these race/cultivar combinations demonstrate quantitative interaction with reversal (Scott *et al.*, 1979) and this accounts for the significant two- and three-way interactions for light, temperature, cultivar and race in the ANOVA (Table 5.3).

For all three parameters of disease, temperature of incubation is the most significant, independant determinant of variation in disease level. Further, for ULD the variance ratios of the two-way interactions involving temperature with race or with cultivar are greater than those of light intensity with race or with cultivar. Finally, the variance of the three-way interaction, temperature X cultivar X race, is higher than that for light intensity X cultivar X race (Table 5.3). Thus, in these cultivar/race combinations both independantly, and through interactions, temperature (range 15 to 25°C) is a more important determinant of variation in ULD than is light intensity (range 100 to 1000 μE $m^{-2}s^{-1}$). However, light intensity (range 50 to 200 μE $m^{-2}s^{-1}$) is a more signifcant determinant, than is temperature (range 15 to 24°C), of variation in

ULD in complexes of three races of *M. larici-populina* and four cultivars of *P. X euramericana* (Chandrashekar and Heather, 1982). While this contrast may result partly from the inclusion in the present analysis of zero (0) values for ULD for certain combinations, particularly those incubated at 25°C/1000 μE $m^{-2}s^{-1}$, it is possible that the physiology of the host/pathogen reaction to combinations of temperature and light intensity differs between the two systems.

The present results demonstrate that the reaction of races of *M. medusae* with cultivars of *Populus* spp. is very labile to variation in factors of the physical environment and that the environmental factors can be as, or even more, important than the race or cultivar constitution in determining disease severity in compatible race/cultivar combinations. This characteristic, which is independant of the artificial nature of the experiment, and which is supported by similar observations in this host/pathogen system (section 4.3, Chapter 4) and in *M. larici-populina/Populus* species system (Chandrashekar, 1981, Chandrashekar and Heather, 1981a,b,c, 1982) should help maintain the stability of disease resistance in such host/pathogen complexes under natural conditions. A further discussion of the results reported here is given in Chapter 9.

CHAPTER 6

SENSITIVITY TO PRE- AND POST-INOCULATION TEMPERATURE OF THE REACTIONS OF *POPULUS* SPECIES TO *MELAMPSORA MEDUSAE*.

INTRODUCTION

Reports of the effect of post-inoculation temperature on disease development in various host/pathogen combinations are frequent (section 4.1, Chapter 4; section 5.1, Chapter 5) while those of the predisposing effect of pre-inoculation temperature (the temperature at which the host plants are raised) are less common (Sharp, 1962a,b; Brown and Shipton, 1964; Chandrashekar and Heather, 1981c). The concepts, definitions and factors of predisposition have been discussed by Yarwood (1959). Temperature has been reported as the most important factor in predisposing plants to diseases (Colhoun, 1979). Reports of the combined effects of pre- and post-inoculation temperature on disease development in compatible hosts are very limited (Chandrashekar and Heather, 1981c).

In an experiment, using races of *M. larici-populina* and cultivars of *P. X euramericana*, the level of leaf rust, as assessed on most parameters, was highest when plants were raised on a low (20/10°C - day/night) temperature regime and incubated, subsequent to inoculation, at a low (20°C) constant temperature. In contrast, leaf

rust was minimal when the cultivars were raised on a high (28/20°C - day/night) temperature regime and incubated, at high (25°C) constant temperature (Chandrashekar and Heather, 1981c).

Results of earlier experiments (section 4.3, Chapter 4; section 5.3, Chapter 5) demonstrated that high (25°C), as compared with low (15°C), post-inoculation temperature was less favourable for both the qualitative and the quantitative development of races of *M. medusae* on cultivars of poplar.

In this chapter the results of an experiment in which leaf disks, cut from compatible, replicate cultivars of poplar, raised on a high (28/20°C - day/night) or a low (20/10°C - day/night) temperature regime, were inoculated with an isolate of *M. medusae* and subsequently incubated at both high (28/20°C - light/dark) and low (20/10°C - light/dark) temperature regimes, are reported. These temperature regimes are typical of those which occur at different periods of the growing season of poplar in southeastern Australia.

6.2 MATERIALS AND METHODS

6.2.1 PLANTS: Replicate plants (actually clones) of compatible cultivars of *P. deltoides* (cvv. *67/PC-7-4, W 79/304* and *W 79/307*) and *P. X euramericana* (cvv. *I-214* and *I-488*) were grown as detailed by Chandrashekar and Heather (1981c) for three months in separate rust-free phytotron cabinets (Morse and Evans, 1962) on day/night temperature regimes of 28/20 and 20/10°C.

6.2.2 INOCULUM: Urediniospores of six races of *M. medusae* (Chapter 4) were multiplied separately on detached leaves of *P. X euramericana* cv. *I-488* (section 2.5.1, Chapter 2), and subsequently thoroughly mixed in equal proportions by weight, to produce at least 60mg of urediniospore inoculum, and used to inoculate the leaf disks.

6.2.3 SELECTION, INOCULATION AND INCUBATION OF LEAF DISKS: The selection of leaves (section 2.3.1, Chapter 2), surface treatment of these (section 2.3.2, Chapter 2) cutting and preparation of leaf disks (section 2.3.3, Chapter 2) for inoculation and deposition of urediniospores on the disks in a spore settling tower (section 2.5.2, Chapter 2) followed the procedures and observed the precautions detailed previously. Replicate leaf disks of each cultivar, raised on each temperature regime, were inoculated in a series of depositions, each employing 5mg of urediniospores, in the spore settling tower. Five leaf disks of each cultivar, raised on each pre-inoculation temperature regime, were randomly allocated and placed on plastic foam saturated with 10 p.p.m. gibberellic acid in each of twenty four divided glass Petri plates (section 2.6.2, Chapter 2). All plates were then incubated at 20°C in the dark for 24h to permit uniform urediniospore germination and penetration of the disks by the fungus (Spiers, 1978; Singh, Unpublished data). This treatment ensured that observations in the experiment resulted from the post-penetration interaction of the host, pathogen and environment and excluded potential effects on spore germination (Appendix 9) of the varying temperatures of incubation. Following this pre-treatment, twelve replicate Petri plates, containing leaf disks from plants of each

cultivar grown at a particular temperature regime were allocated for post-inoculation incubation at temperature regimes of 28/20 and 20/10°C - light/darkness, 16h photoperiod, cool, fluorescent light, intensity 100 μE $m^{-2}s^{-1}$ in separate L.B. phytotron cabinets (Morse and Evans, 1962). For brevity, the combinations of pre- and post-inoculation temperature treatments are referred to as: High-high (28/20-28/20°C), High-low (28/20-20/10°C), Low-high (20/10-28/20°C) and Low-low (20/10-20/10°C).

6.2.4 DATA RECORDED: The leaf disks were examined daily and disease development recorded on the following parameters: Incubation period (days) to flecking (IPF), latent period (days) to eruption of the first uredinium (LP1), latent period (days) to production of 50 per cent of the number of uredinia recorded at 14 days of incubation (LP50), and number of uredinia per leaf disk after incubation for 14 days (ULD).

6.2.5 STATISTICAL ANALYSIS: The data for all the parameters were tested for normality and homoscedasticity using a GLIM programme (Nelder, 1975).

6.2.5.1 ANALYSIS OF VARIANCE: Data were analysed using the sub-programme ANOVA of the SPSS (Nie *et al.*, 1975). Paired comparisons between cultivars and levels of treatments were tested for statistical significance (P = 0.01) using LSD (Steel and Torrie, 1960).

6.2.5.2 REGRESSION ANALYSIS: A linear regression model was employed to assess co-efficients of determination between the pairs of parameters used to assess disease intensity. These co-efficients were computed for the overall data, for individual cultivars across all temperature combinations, and for individual temperature combinations across all cultivars.

6.3 RESULTS

Plants grown at high (28/20°C), as compared to low (20/10°C), pre-inoculation temperature were taller and bore more but thinner leaves. At low temperature, plants of cultivars of *P. X euramericana* were taller than those of cultivars of *P. deltoides* while growth of all the cultivars was comparable at high pre-inoculation temperature.

Data on disease severity, as assessed on various parameters, developed in the cultivars of *Populus* grown at high (28/20) or low (20/10°C) temperature, inoculated with urediniospores of *M. medusae*, and subsequently incubated at high (28/20°C) and low (20/10°C) temperature, are presented in Table 6.1 and Figure 6.1.

The major components (pre- and post-inoculation temperature and cultivars) and most of their second and third degree interactions are significant ($P = 0.001$) contributors to variation in disease intensity as assessed on the four parameters (Table 6.2). Since a fixed model was employed in the ANOVA, the degrees of freedom and sum of squares for the interaction terms can be added to those for the appropriate residual, and the variance ratios for the major terms

Table 6.1 Disease intensity (assessed on four parameters) developed in five replicated cultivars of *Populus* spp., raised on a high and a low pre-inoculation temperature† regimes inoculated with *M. medusae* and subsequently incubated on high and low temperature regimes†

Cultivar	Disease parameter	Pre-inoculation Temp. °C (Day/Night) 20/10		28/20		Overall Means
		Post-inoculation Temp. °C (Light/Dark) 20/10	28/20	20/10	28/20	
I-214	IPF*	**5.03c	4.05hij	6.15a	4.50ef	4.94q
	LP1*	7.12efg	6.17l	7.25cde	6.50k	6.76p
	LP50*	7.88hi	7.17j	8.07gh	7.30j	7.61p
	ULD*	25.10hijk	20.74ijk	29.18ghi	26.50ghij	25.42q
I-488	IPF	4.77cde	3.78jk	5.38b	4.03hij	4.49p
	LP1	7.47bc	6.61jk	7.57ab	6.72ijk	7.09r
	LP50	8.15fgh	7.70i	8.15fgh	7.32j	7.83q
	ULD	22.40ijk	18.29jk	17.29jk	15.08k	18.27p
7-4	IPF	5.53b	4.15gh	5.98a	4.38fg	5.01q
	LP1	7.72a	6.76hij	7.35bcde	7.22de	7.27s
	LP50	10.00a	8.64bc	8.48cde	8.81b	8.99s
	ULD	35.83fg	40.75ef	43.05ef	37.12b	39.63r
79/304	IPF	4.68de	3.47l	5.65b	4.15gh	4.49p
	LP1	7.12efg	6.19l	7.53ab	6.98fgh	6.96q
	LP50	8.56bcd	7.74i	8.29efg	8.48cde	8.27r
	ULD	112.12a	91.02b	50.51e	48.37e	75.04t
79/307	IPF	4.90cd	3.52kl	5.98a	4.10ghi	4.62p
	LP1	7.13efg	6.90ghi	7.20def	7.37bcd	7.15r
	LP50	8.50cde	8.20fg	8.07gh	8.37def	8.29r
	ULD	89.70c	42.38ef	66.58d	33.32fgh	58.10s
Overall Means for temperature combinations	IPF	4.99y	3.79w	5.83z	4.23x	
	LP1	7.31y	6.53w	7.38z	6.96x	
	LP50	8.62z	7.89w	8.21y	8.06x	
	ULD	57.03y	42.64x	41.32x	32.08w	

†Pre-inoculation temperature regimes (High - 28/20°C - day/night)
(Low - 20/10°C - day/night)
Post-inoculation temperature regimes (High - 28/20°C - light/dark)
(Low - 20/10°C - light/dark)

*IPF - incubation period to flecking, LP1 - latent period to production of the first uredinium, LP50 - latent period to production of 50 per cent of uredinia, ULD - number of uredinia per leaf disk at 14 days after inoculation.

**Each value in the body of the table is the mean of *ca.* 60 leaf disks

For any disease parameter, individual values sharing the same superscript, do not differ significantly ($P = 0.01$)

For any disease parameter, overall mean values sharing the same superscript, do not differ significantly ($P = 0.01$).

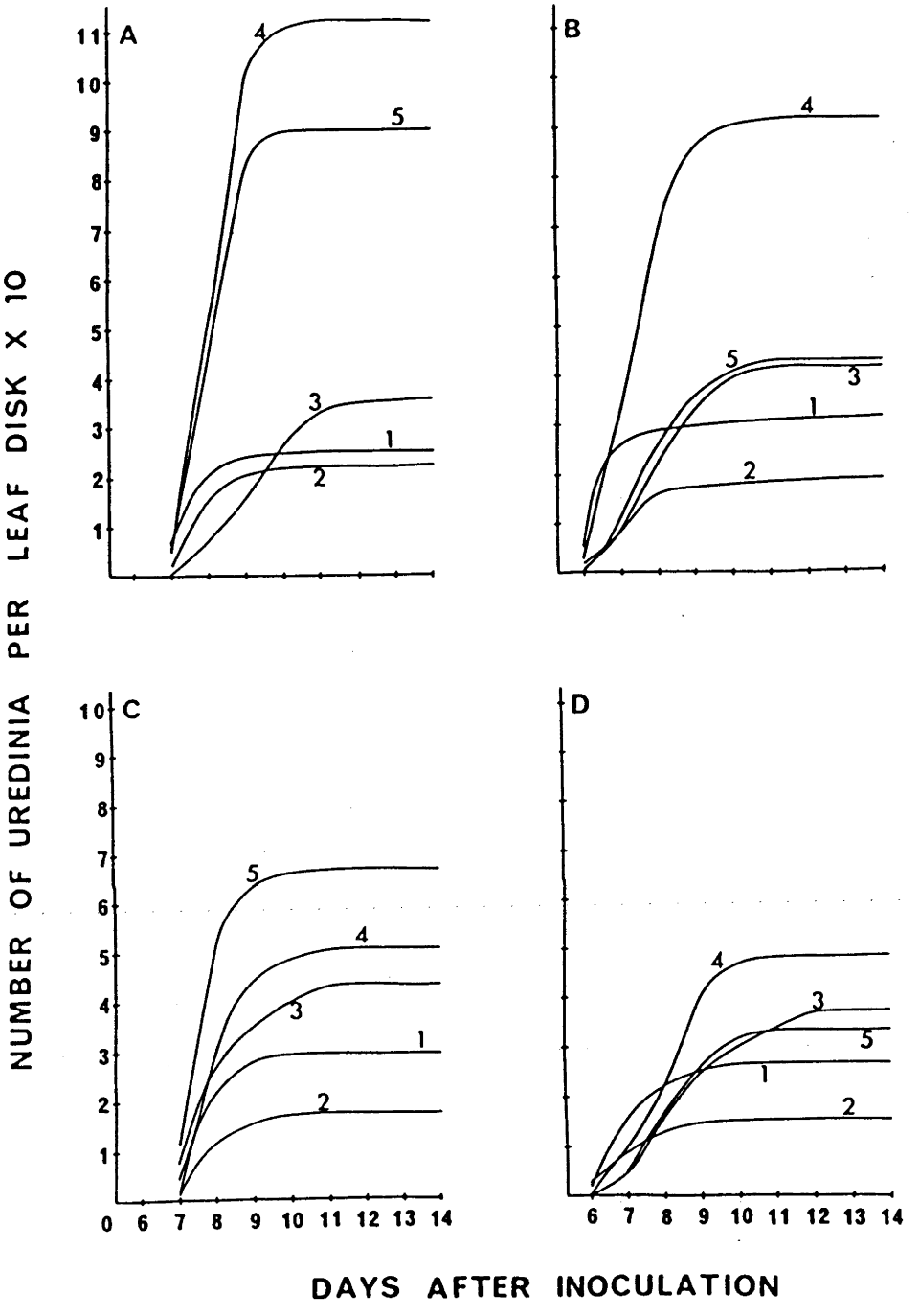

Figure 6.1 Disease progress curves (ULD) for five cultivars (1, *I-214*; 2, *I-488*; 3, *7-4*; 4, *W 79/304* and 5, *W 79/307*) of *Populus* spp. in different combinations of pre- and post-inoculation temperature (A, 20/10-20/10; B, 20/10-28/20; C, 28/20-20/10 and D, 28/20-28/20°C) treatments.

Table 6.2 Analysis of Variance of four parameters of disease intensity developed on five cultivars of *Populus* grown at two pre-inoculation temperature regimes, inoculated with *M. medusae* and subsequently incubated on two post-inoculation temperature regimes.

Source of Variation	df	Disease parameter IPF		LP1		LP50		ULD	
		Mean Square	F-ratio	Mean Square	F-ratio	Mean Square	F-ratio	Mean Square	F-ratio
PRT	1	123.106	356.406	18.497	80.897	4.368	15.109	52118.971	109.733
PST	1	574.631	1663.627	107.053	468.188	58.091	200.919	42314.806	89.091
CV	4	14.706	42.575	9.012	39.412	66.448	229.821	130361.132	274.466
PRT X PST	1	11.984	34.696	9.742	42.607	24.450	84.566	2158.928	4.545**
PRT X CV	4	3.370	9.757	2.763	12.085	7.647	26.448	31148.434	65.581
PST X CV	4	1.905	5.514	7.012	30.666	5.157	17.838	15863.026	33.398
PRT X PS TX CV	4	0.478	1.383NS	1.415	6.190	10.182	35.215	1783.016	3.754*
Residual	1167	0.345		0.229		0.289		474.963	
Total	1186								

Abbreviations: IPF, LP1, LP50 and ULD are described in Table 6.1.

Abbreviations: PRT = pre-inoculation temperature regime; PST = post-inoculation temperature regime; CV = cultivar, and df = degrees of freedom.

NS = Variance ratio is not significant.
* = Variance ratio significant at $P = 0.05$.
** = Variance ratio significant at $P = 0.03$.
All unscored variance ratios are significant at $P = 0.001$.

re-calculated (section 2.10.1, Chapter 2). When this procedure is followed, all the major components are still significant ($P = 0.05$,) contributors to the total variance for each parameter of disease and thus their individual contributions to variation can be discussed.

Post-inoculation temperature is the most and the cultivar the least important contributor to variation in IPF and LP1 while cultivars are the most significant contributors to variation in LP50 and ULD (Table 6.2).

For the overall data the high, when compared with the low, post-inoculation temperature regime decreased IPF, LP1, LP50 and ULD (mean values, Table 6.1). In contrast high, when compared with low, pre-inoculation temperature usually increased IPF, LP1 and LP50 but decreased ULD (mean values, Table 6.1). Certain cultivars, at particular combinations of the pre- and post-inoculation temperature regimes, do not follow this generalised pattern for all parameters. However, for the individual cultivars high, when compared with low, post-inoculation temperature always resulted in decreased IPF and usually decreased ULD, while high, when compared with low, pre-inoculation temperature always increased IPF and usually reduced ULD (Table 6.1).

The effect of the combinations of pre- and post-inoculation temperature regimes on disease intensity as determined by the four parameters is summarised for the overall data in Figure 6.2. On the basis of ULD (the type of parameter most commonly used to assess

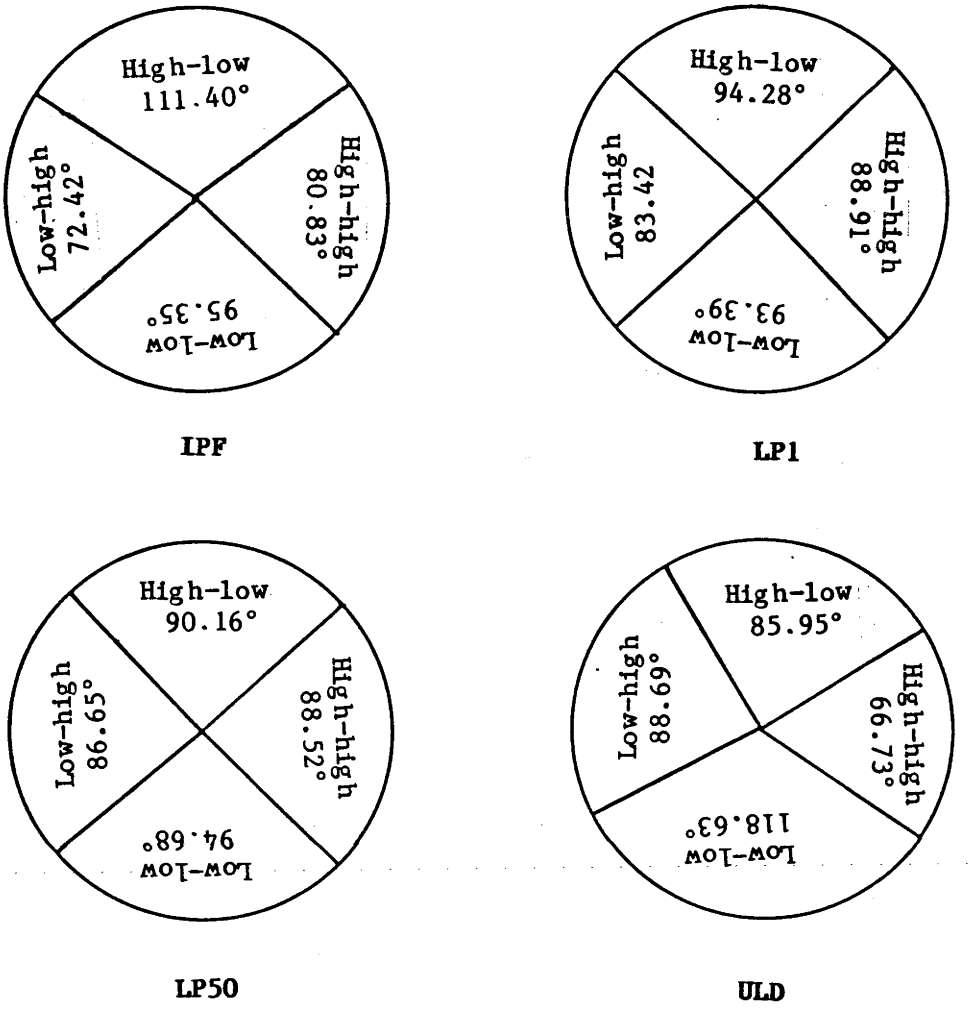

Figure 6.2 The proportion (assessed in degrees) which combinations of pre- and post-inoculation temperature make to disease intensity as assessed on four parameters: IPF - incubation period to flecking , LP1 - latent period to production of first uredinium, LP50 - latent period to production of 50 per cent uredinia, ULD - number of uredinia per leaf disk at 14 days of incubation.

disease intensity in the leaf rusts), when averaged over all cultivars, the Low-low combination resulted in the highest, while the High-high caused the lowest, level of disease. IPF and LP1 are shortest in the Low-high and longest in the High-low while LP50 is shortest in the Low-high and longest in the Low-low combination of pre- and post-inoculation temperature. The significant third order interaction for most parameters of disease in the ANOVA (Table 6.2) indicates that the clarity of these overall patterns varies within some cultivars.

The ranking of the cultivars for relative resistance (longer IPF, LP1, LP50 and lower ULD characterise higher resistance) is somewhat dependant on the parameter of disease assessment employed, and the combination of pre- and post-inoculation temperature regimes in which the interaction occurs (Table 6.1, Figure 6.2). This dependance is confirmed by the high significance of most of the second and the third order interactions for most parameters in the ANOVA (Table 6.2). However for most parameters, but specifically for ULD, under most temperature combinations, *W 79/304* is the most, and *I-488* the least, susceptible cultivar.

The assessment of co-efficients of determination between pairs of the parameters employed to monitor disease intensity in the overall data (results combined across cultivars and temperature treatments) demonstrates that the parameters are poorly correlated (Table 6.3). This is confirmed by comparisons of the contribution which the temperature combinations make to the total variation for each parameter of disease (Figure 6.2). In certain instances, the

Table 6.3 Coefficient of determination† between paired parameters employed to measure disease intensity induced on five cultivars of *Populus*, raised on a high and a low pre-inoculation temperature regimes, inoculated with an isolate of *M. medusae* and subsequently incubated on a high and a low post-inoculation temperature regime.

Pairs of disease intensity parameters	Variance ratio	Coefficient of determination(R^2)
IPF* and LP1	16.10**	0.47
IPF and LP50	2.16	0.11
IPF and ULD	0.00	0.00
LP1 and LP50	16.00**	0.47
LP1 and ULD	0.24	0.01
LP50 and ULD	1.94	0.10

† Linear model

* IPF - incubation period to flecking; LP1 - latent period to formation of first uredinium; LP50 - latent period to production of 50 per cent of uredinia; ULD - number of uredinia per leaf disk.

Variance ratios scored by ** are significant at $P = 0.005$;

Unscored ratios are not significant at $P = 0.005$.

correlations between parameters are improved when the data analysed are restricted to either a single cultivar across all temperature combinations or to a single temperature combination across all cultivars (Appendix 10). For example for cultivar *I-488* the co-efficients of determination between IPF and LP1, and LP50 and ULD are 0.93 and 0.70 respectively. Similarly, for the High-high temperature combination the co-efficient of determination between LP50 and ULD averaged across all cultivars is 0.65.

6.4 DISCUSSION

The germination of urediniospores of *M. medusae* is depressed significantly by incubation at 25 rather than 15°C (Appendix 9). The design of the present experiments eliminated the potential effects of post-inoculation temperature on germination of urediniospores and subsequent penetration of the host by the fungus. The results of these experiments demonstrate that pre- and post-inoculation temperature, and their combinations, affect significantly the post-penetrative interaction of cultivars of poplar with *M. medusae*. Extrapolation to the probable epidemiology of leaf rust in the field requires caution since, apart from the artificiality of laboratory investigations, these experiments cover only a portion of a typical disease mono-cycle.

The ranking of the cultivars for relative disease resistance is disease parameter and temperature regime dependant (Table 6.1). When ranked on the basis of ULD the segregation is frequently clear-cut

(cultivars differ significantly in disease level), and relatively uniform, over the various temperature regimes (Table 6.1, Figure 6.1). Despite this general qualitative uniformity, the significant second degree interactions in the ANOVA (Table 6.2) demonstrate a degree of differential interaction of cultivars and temperature regimes at a quantitative level. These relationships agree with those demonstrated between cultivars of *Populus* and *M. medusae* (section 4.3, Chapter 4; section 5.3, Chapter 5) and *M. larici-populina* (Chandrashekar and Heather, 1981a,c).

The high pre-inoculation temperature regime always predisposed the cultivars to a greater resistance on the basis of IPF and LP1 and ULD (Table 6.1, Figure 6.1). This observation agrees with that of Chandrashekar and Heather (1981c) for *M. larici-populina* on poplar and those for *Puccinia striiformis* West. on some (Gassner and Straib, 1934; Newton and Johnson, 1936), but not all (Sharp, 1962a,b), varieties of wheat. In contrast, exposure of the wheat variety *Little Club* to a low temperature regime, prior to inoculation with *Puccinia graminis* f.sp. *tritici*, resulted in reduced penetration by urediniospores (Brown and Shipton, 1964).

The high, when compared with low, post-inoculation temperature regime resulted in a lower ULD. This agrees with the observations of Toole (1967), Spiers (1978), and (section 4.3, Chapter 4; section 5.3, Chapter 5) for *M. medusae* and with that of Chandrashekar and Heather (1981a,c) for *M. larici-populina*.

The shorter IPF, LP1 and LP50 in the high, than in the low, post-inoculation temperature regime suggest that high post-

inoculation temperature increases the susceptibility of the cultivars to *M. medusae* leaf rust. This result contrasts with the conclusion, based on ULD, that the high post-inoculation temperature regime resulted in higher resistance. This conflict probably results, as has been suggested (Chandrashekar and Heather, 1981c; Sharp *et al.*, 1958), because the temperature regimes affect differentially the processes leading to symptom production and fructification.

Since the length of the LP1 includes the period of the IPF, and the LP50 is determined in part by the ULD, it had been expected that the co-efficients of determination between IPF and LP1 and between LP50 and ULD would have been more pronounced than demonstrated here (Table 6.3). Further, observations on leaf rust in barley demonstrated a high inverse correlation between LP50 and ULD (Parlevliet, 1975) and a satisfactory positive correlation was shown between IPF and LP1 with leaf rust caused by *M. larici-populina* on two cultivars of *Populus* (J. K. Sharma *et al.*, 1980). Possibly this conflict results because these other investigations included the effects of temperature on both pre- and post-penetration phenomena while the present experiments investigated only post-penetrative relationships.

Since IPF is capable of rapid and accurate (*sensu* low residual variance in the ANOVA) determination of host/pathogen reactions, its capacity to predict ULD is worthy of considerable further investigation. While the parameters employed to measure disease intensity in this investigation are useful to describe aspects of the host/pathogen relationship, they probably lack epidemiological

significance and hence predictive value for disease modelling. Decreases in IPF, LP1 and LP50 reduce the period between crop infection and first appearance of disease in an epidemic (van der Plank, 1963, 1968). However, since such decreases also reduce the length of individual mono-cycles they increase the value of 'r^1' (rate of disease increase) in such epidemics, i.e., the slope of the linear portion of the sigmoidal curve is increased. Increases in ULD, or a comparable parameter, in a mono-cycle also increase the value of 'r^1' in an epidemic. Thus a measure of disease level (e.g. disease progress curves, Figure 6.1) which includes all those parameters may have more epidemiological significance (Kranz, 1974a,b).

Growth of plants (e.g. size and thickness of leaves, section 6.3, *ibid.*) raised at high and low pre-inoculation temperature regimes and their susceptibility to *M. medusae* varied in the present experiments. Pre-inoculation temperature is reported to affect the morphology and physiology of plants and to predispose these to diseases (Ayers, 1978). Thus comparison of morphology and physiology of plants grown at different pre-inoculation temperatures might explain the differences in the susceptibility of plants in the present experiments.

Extrapolation from the present results, obtained from the experiments in the laboratory conducted under controlled conditions, to possible effects of pre- and post-inoculation temperature on the development of *M. medusae* leaf rust in the field must be very tentative. In the field through a growing season, the leaves on plants of different cultivars emerging at different dates could be

differentially predisposed because the shoots are subjected to diurnal and seasonal fluctuations in the temperature. Additionally, the possibility of interaction between pre- and post-inoculation temperature and other environmental factors would complicate the understanding of the effects of temperature on disease development. Thus the susceptibility of a particular leaf in the crown at any time in the growing season would depend on the complex of genetic, ontogenetic and environmental factors. The present results suggest that in the field comparable leaves developed at high temperature, infected and exposed to a high temperature will be more resistant than those developed, infected and exposed in a low temperature regime. Hence, as suggested by Chandrashekar and Heather (1981c) for *M. larici-populina* leaf rust in Australia, within the limits imposed by genetic and other factors, infections of poplar leaves which occur in early/late spring rather than in summer, are more likely to cause epidemics of *M. medusae* leaf rust in the field.

CHAPTER 7

EFFECTS OF PRE- AND POST-INOCULATION PHOTOPERIOD ON THE SEVERITY OF *MELAMPSORA MEDUSAE* LEAF RUST OF *POPULUS* SPECIES.

7.1 INTRODUCTION

Populus species occur naturally, and are cultivated, over a wide range of latitude (Anon., 1979). Certain cultivars of *Populus* species are day-length sensitive and hence their cultivation is restricted to particular latitudes (Pryor and Willing, 1965), however, the effect of photoperiod on the *Populus/Melampsora* leaf rust system has not been reported. Bever (1934) demonstrated that the incubation period of *Puccinia glumarum* (Schm.) Erikss. & Henn. on cultivar *Panier* of barley doubled with an increase in the photoperiod from 6 to 12h per day; further under a 15h photoperiod, or in continuous light, inoculated barley plants developed infection type 0 (extremely resistant), but infection type 4 (completely susceptible), when incubated on a photoperiod of 12h or less. The leaves and stems of the wheat cultivar *Hope* were more susceptible to race 21 of *Puccinia graminis* f.sp. *tritici* when raised, and incubated, subsequent to inoculation, on a photoperiod of 6h, than when grown and incubated on a 10h photoperiod or in full day-light (Johnson and Newton, 1940). Tomato cultivar *Bonny Best* raised on short (6h) pre-inoculation photoperiod was more susceptible to *Fusarium* wilt than when grown on

long (18h) photoperiod (Foster and Walker, 1947); this cultivar was also more susceptible to *Verticillium* wilt when cultured on a short (4h) rather than a longer (8+ h) photoperiod (Jones *et al.*, 1975). Pegg (1981) has cited other examples of the effect of long or short days on susceptibility of crop plants to wilt diseases.

Increasing light intensity in the range 100 to 1000 μE $m^{-2}s^{-1}$ is inhibitory to the germination of urediniospores of *M. medusae* (Appendix 9), and inoculated leaf disks of *Populus* species incubated over this range demonstrated lower infection type (Chapter 4) and reduced number of uredinia per unit area (Chapter 5) with increasing light intensity.

This chapter reports the effects of combinations of two pre-inoculation and two post-inoculation photoperiods on the severity of infection of leaf disks of four cultivars of *Populus* spp. inoculated with race 4 of *M. medusae*. The photoperiods employed in this experiment approximate the maximum and minimum day-length experienced in the Canberra area (Latitude 35°S).

7.2 MATERIALS AND METHODS

The general procedures of raising plants (section 2.2.3), multiplication of mono-urediniospore isolates (section 2.4.2) and preparation (section 2.3.3), inoculation (section 2.5.2) and incubation (section 2.6.2) of leaf disks are described in the foregoing Chapter 2

7.2.1 GROWTH OF HOST MATERIAL: Replicate cloned plants of four cultivars of *Populus* spp. (*P. X euramericana* cvv. *I-214, I-488* and *P. deltoides* cvv. *W 79/304, W 79/307*) were raised in separate L.B. phytotron cabinets (section 2.2.3, Chapter 2) on photoperiods of 10 and 15h (per 24h) under comparable conditions of light (cool, fluorescent tubes, Philips TL MF 140W/33 RS double flux, intensity 350 μE $m^{-2}s^{-1}$ at the pot surface), and temperature (25°C, 14h and 15°C, 10h, in each 24h cycle). Thus, the plants were subjected to 25°C for all (10h photoperiod) or most (15h photoperiod) of their light period.

7.2.2 INOCULUM: Urediniospores (mono-urediniospore origin) of race 4 of *M. medusae* were multiplied to obtain at least 20mg of dried inoculum (section 2.4.1, Chapter 2). The use of more than one race in this experiment would have resulted in practical difficulties in replication. Of the races available race 4 was selected because, 1. most of the races produced reactions identical to those of race 4 (Table 4.1, Chapter 4) and, 2. this race, as compared to others was moderately aggressive and relatively more stable at different combinations of temperature and light intensity of incubation (Table 5.2; Figure 5.2, Chapter 5).

7.2.3 INOCULATION: Ten leaf disks cut from each cultivar, raised on each of the two photoperiods, were included in a series of four successive inoculations. Inoculation of these leaf disks with 5mg lots of urediniospores in a spore settling tower followed procedures detailed elsewhere (section 2.5.2, Chapter 2). Some cover glasses

were included in each inoculation to estimate the number of urediniospores deposited (Appendix 8) per unit area and the germination potential (Appendix 9) of the urediniospores employed. To obtain sufficient inoculated replicate disks of all cultivars, raised on the two pre-inoculation photoperiods, for incubation at the two post-inoculation photoperiods, it was necessary to repeat the inoculation four times. Deposition of urediniospores in the successive inoculations did not differ significantly (P = 0.01) from that within an inoculation (1.86±0.07 urediniospores per mm^2). Some additional cover glasses were included at each inoculation and following incubation at 20±1°C in the dark for 24h, the germination of urediniospores on these exceeded 92 per cent.

7.2.4 INCUBATION: Following inoculation, randomly selected groups of five leaf disks of each cultivar grown at each pre-inoculation photoperiod were located on plastic foam soaked in 10 p.p.m. (μg/ml) gibberellic acid in separate compartments within 14cm glass Petri plates (section 2.6.2, Chapter 2) for incubation (20±1°C and light intensity of 100 $\mu E\ m^{-2}s^{-1}$) on a photoperiod of either 10 or 15h per 24h cycle. Thus, after the four inoculations, twenty replicate leaf disks of each cultivar, raised at each of the pre-inoculation photoperiod treatments, were incubated at each post-inoculation photoperiod.

7.2.5 ASSESSMENT OF DISEASE SEVERITY: Individual leaf disks were examined daily over the 12 day incubation period and disease development recorded using the following parameters: IPF - incubation

period (days) to flecking (flecks, localized chlorotic areas are the first visible symptoms of disease development); LP1 - latent period (days) to eruption of the first uredinium; LP50 - latent period (days) to eruption of 50 per cent of the uredinia recorded after 12 days of incubation; ULD - number of uredinia per leaf disk after 12 days of incubation; USM - number of urediniospores produced per mm^2 of leaf disk surface after 12 days of incubation; USU - number of urediniospores produced per uredinium after 12 days of incubation. For calculation of USM and USU after 12 days of incubation, leaf disks, in groups of five, were transferred to 10ml solution of 1 per cent agar containing 10 drops per litre of Tween-20 in labelled McCartney bottles. The urediniospores were dislodged into the solution by vigorous shaking for 45 minutes on a mechanical shaker. Haemocytometer counts on the resulting suspension were used to calculate USM (section 2.8.2.8, Chapter 2), and USU (section 2.8.2.9, Chapter 2) was computed by combining this reading with that for ULD.

7.2.6 STATISTICAL ANALYSIS:

The data for all the parameters of disease assessment were tested for homoscedasticity and normality (Neter and Wasserman, 1974) using a GLIM programme (Nelder, 1975).

7.2.6.1 ANALYSIS OF VARIANCE: The data were analysed using the sub-programme ANOVA of SPSS (Nie *et al.*, 1975). For all combinations of pre- and post-inoculation photoperiods data of disease severity, assessed by each parameter, were averaged across cultivars and LSD

(P = 0.01) (Steel and Torrie, 1960) between cultivars, and between photoperiod combinations, were calculated.

7.2.6.2 STATISTICAL COMPARISONS OF DISEASE PROGRESS CURVES: Paired comparisons of curves of disease progress (basis number of uredinia per leaf disk), over time (readings commenced in all instances from the date of the eruption of the first uredinium), for cultivars in each combination of pre- and post-inoculation photoperiod, were made using standard analysis of variance techniques (Rao, 1952) as described by Sharma *et al.* (1980). The residual sums of squares of the fitted curves for a particular pair of cultivars in a particular combination of pre- and post-inoculation photoperiod were added to obtain a total residual sum of squares (r_1) with f_1 degrees of freedom. Then a single curve was fitted to the combined data for the particular pair of cultivars to yield a residual sum of squares (r_2) with f_2 degrees of freedom. The difference $r_0=r_2-r_1$ (with $f_o=f_2-f_1$ degrees of freedom) is the sum of squares of residuals due to deviation from the hypothesis that the curves are the same (the null hypothesis). The curves were fitted using a GLIM programme (Nelder, 1975) and employing the cubic model:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + E_i$$

7.3 RESULTS

Plants grown in the 15h pre-inoculation photoperiod were obviously taller and carried more, and larger, leaves than those raised on the

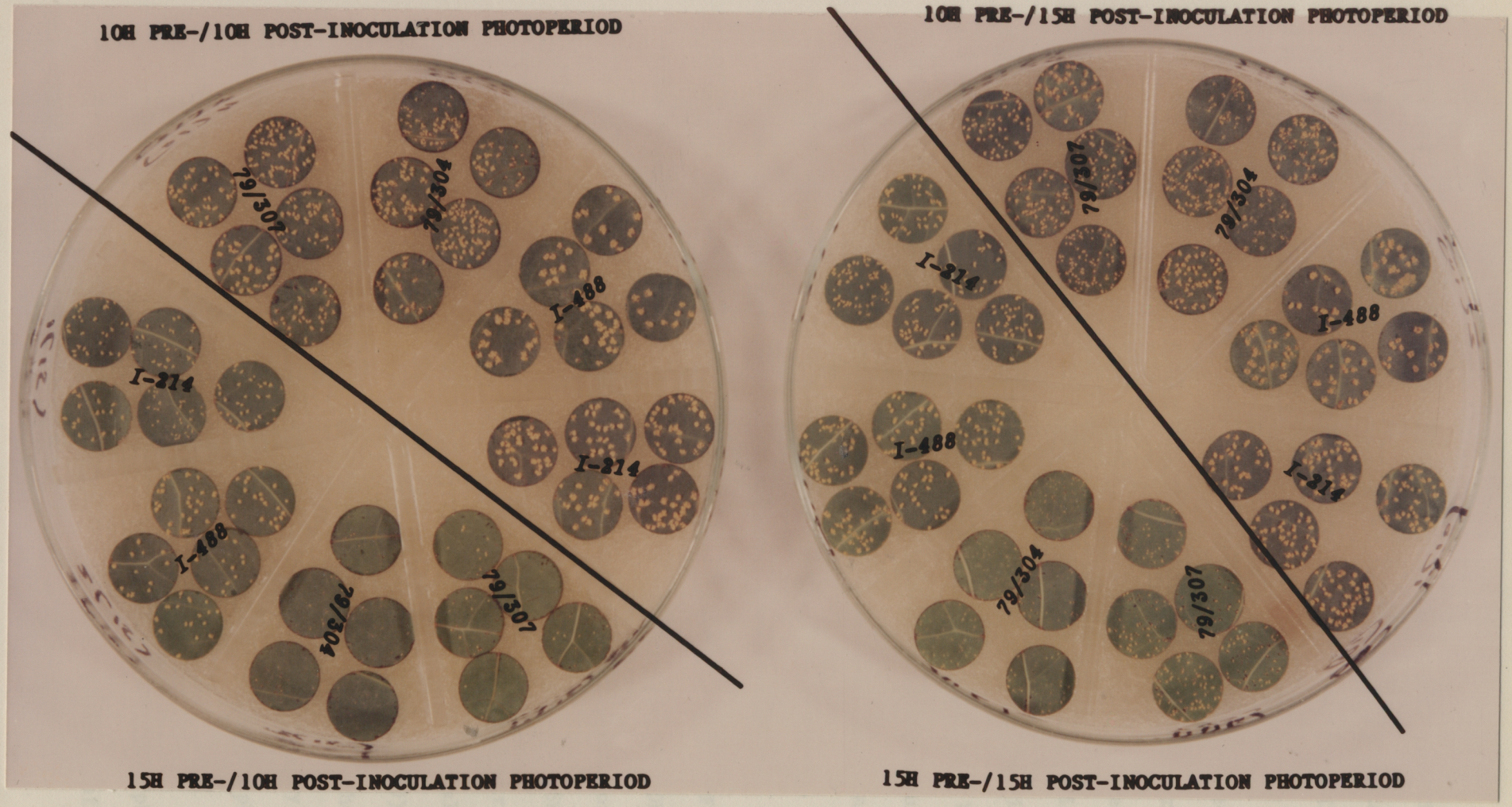

Plate 7 Comparison of disease reactions developed in four cultivars of poplar, raised at two pre-inoculation photoperiods and incubated, subsequent to inoculation with urediniospores of race 4 of *M. medusae*, at two post-inoculation photoperiods.

Irrespective of the combination of pre- and post-inoculation photoperiod, the uredinia developed on cultivars of *P. X euramericana* were larger than those produced on cultivars of *P. deltoides* (Table 7.1 and 7.2, refer to USU values; Plate 7). Plants of cultivars (especially those of *P. deltoides*) raised at long, as compared to short, photoperiod supported a lower number of uredinia per leaf disk and these uredinia were comparatively smaller in size when compared with other cultivars (Table 7.1 and 7.2, refer to USU values; Plate 7). *P. deltoides* cv. *W 79/304*, when raised at long pre-inoculation photoperiod developed fewer uredinia per leaf disk at both post-inoculation photoperiods (Table 7.1). In contrast, this cultivar was relatively highly susceptible (as assessed on ULD) when raised under the short pre-inoculation photoperiod (Table 7.1; Plate 7).

When averaged across the cultivars, disease severity induced in the leaf disks is generally greatest (shortest mean IPF and LP1, highest mean ULD, USM and USU) with the combination of 10h pre- and 15h post-inoculation photoperiods, however, on the basis of shortest LP50 disease severity is highest in the combination of 15h pre- and 10h post-inoculation photoperiod (Table 7.3). In contrast, when averaged across all cultivars, disease severity is usually lowest (longest mean IPF and LP1, lowest mean ULD, USM and USU) with the combination of 15h pre- and 10h post-inoculation photoperiod, however, on the basis of longest mean LP50 disease severity is lowest in the combination of 15h pre- and 15h post-inoculation photoperiod (Table 7.3).

When averaged across the pre- and post-inoculation photoperiod treatments the relative resistance, expressed by the leaf disks of

Table 7.1 Disease severity, (six parameters[A]), developed in four cultivars of poplar, raised at two pre-inoculation photoperiods and incubated, subsequent to inoculation with urediniospores of race 4 of *M. medusae*, at two post-inoculation photoperiods.

Cultivar	Disease parameter	Photoperiod Pre-inoculation 10 hour: Post-inoculation 10 hour	10 hour: Post-inoculation 15 hour	15 hour: Post-inoculation 10 hour	15 hour: Post-inoculation 15 hour
I-214	IPF	3.05bc	3.00c	3.30b	3.10bc
	LP1	6.00c	6.00c	6.00c	6.00c
	LP50	6.00d	6.20d	6.00d	6.20d
	ULD	28.65ef	44.45cd	28.75ef	39.40de
	USM	1610.44cd	2727.27a	433.24f	1457.74d
	USU	9893.00b	10798.50b	2652.00fg	6511.50c
I-488	IPF	3.10bc	3.00c	3.30b	3.20bc
	LP1	6.00c	6.00c	6.00c	6.00c
	LP50	6.05d	6.25d	6.40d	6.30d
	ULD	17.00fg	17.60fg	25.80f	40.20de
	USM	962.36e	2036.58b	591.26f	1566.05cd
	USU	9963.00b	20365.75a	4033.50def	6856.25c
79/304	IPF	3.00c	3.00c	3.80a	3.80a
	LP1	6.40b	6.00c	7.45a	7.25a
	LP50	8.10b	9.25a	7.20c	8.90a
	ULD	27.60ef	63.75b	9.90g	21.50fg
	USM	571.73f	1718.75c	39.06g	110.09g
	USU	3645.75def	4745.25d	694.25h	901.25h
79/307	IPF	3.00c	3.00c	3.70 e	3.70a
	LP1	6.00c	6.00c	6.50b	6.00c
	LP50	6.45d	6.40d	6.05d	7.10c
	ULD	47.40cd	78.05a	26.25f	55.05bc
	USM	896.66e	1937.15b	58.59g	472.30f
	USU	3329.50ef	4368.00de	392.50h	1510.00gh

[A] For details of the parameters see text (section 7.2.5).

Table 7.2 Mean disease severity, (six parameters [A]), induced in four cultivars of poplar grown at two pre-inoculation photoperiods and incubated, subsequent to inoculation with urediniospores of race 4 of M. medusae, at two post-inoculation photoperiods

Parameter of Disease	Cultivar I-214	I-488	79/304	79/307
IPF	3.11x [B]	3.15x	3.40y	3.35y
LP1	6.00x	6.00x	6.78z	6.14y
LP50	6.10w	6.25x	8.36z	6.50y
ULD	35.31y	25.15w	30.69x	51.69z
USM	1557.17z	1289.06y	609.91w	841.18x
USU	7463.75y	10304.63z	2496.63x	2400.00x

[A] for details of parameters see text (section 7.2.5).

[B] For a particular parameter, values followed by the same letter do not differ significantly (P = 0.01).

Table 7.3 Disease severity, (six parameters[A]), developed in four cultivars of poplar, raised at two pre-inoculation photoperiods and incubated, subsequent to inoculation with urediniospores of race 4 of *M. medusae*, at two post-inoculation photoperiods.

Parameter of Disease	Pre-inoculation Photoperiod			
	10 hour		15 hour	
	Post-inoculation Photoperiod		Post-inoculation Photoperiod	
	10 hour	15 hour	10 hour	15 hour
IPF	3.04p [B]	3.00p	3.53r	3.45q
LP1	6.10q	6.00p	6.49s	6.31r
LP50	6.65q	7.03r	6.41p	7.13r
ULD	30.16q	50.96s	22.68p	39.04r
USM	1010.30r	2104.94s	280.54p	901.55q
USU	6707.81r	10069.38s	1943.06p	3944.75q

[A] For detalis of parameters see text (section 7.2.5).

[B] For a particular parameter, values followed by the same letter do not differ significantly (P = 0.01).

Table 7.4 Analysis of variance (six parameters [A]) of disease recorded in four cultivars of *Populus* spp. grown at two pre-inoculation photoperiod regimes and incubated, subsequent to inoculation with urediniopores of race 4 of *M. medusae*, under two post-inoculation photoperiod regimes

Source of variation	Variance ratio					
	IPF	LP1	LP50	ULD	USM	USU
Pre-in. photo. (Pre)	172.66	42.60	1.03ns	33.06	1076.87	799.76
Post-in. photo. (Post)	2.49ns	6.94s	64.69	121.16	848.18	194.01
Cultivar	16.07	46.03	242.42	45.88	211.05	408.82
Pre X Post	0.28ns	0.64ns	6.23s	1.73ns	64.64	12.47s
Pre X Cultivar	21.07	24.91	7.92	36.89	39.27	65.32
Post X Cultivar	0.85ns	2.32ns	20.80	8.88	14.61	49.98
Pre X Post X Cultivar	0.28ns	2.18ns	5.23s	5.44s	16.13	33.49

s - significant at $P = 0.01$, ns - non-significant, unscored variance ratios are significant at $P = 0.001$.

[A] - For details of the parameters see text (section 7.2.5).

the cultivars, is dependent on the parameter employed for disease assessment, e.g., on the basis of the longest mean IPF, LP1 and LP50, *P. deltoides* cv. *W 79/304* is the most resistant cultivar, but *P. X euramericana* cv. *I-488* or *P. deltoides* cv. *W 79/304* is the most resistant cultivar when assessed on the basis of lowest mean ULD or the lowest mean USM respectively (Table 7.2).

In the data analysis, a fixed effect model ANOVA was employed (Table 7.4). Thus, if the value of the residual variance is adjusted to take account of that due to the interactions, and the variances of the major factors re-calculated, the significance of these can be discussed independently despite the significance of the interaction variances in the ANOVA (section 2.10.1, Chapter 2). When this re-calculation is made, the cultivars and the pre- and the post-inoculation photoperiods are significant (P = 0.01) determinants of variation in the disease severity as assessed for most parameters. The obvious exceptions are the effect of post-inoculation photoperiod on IPF and LP1 and of pre-inoculation photoperiod on LP50.

The relative importance of the major factors, i.e., cultivar, pre-inoculation photoperiod and post-inoculation photoperiod, as determinants of variation in the parameters of disease severity depends on the parameter employed, e.g., pre-inoculation photoperiod is the most important independant determinant of IPF, USM and USU but is not a significant cause of variation in LP50; while the cultivar is the outstanding determinant of variation in LP50, and LP1 but a minor determinant of USM. There are comparable variations in the importance of the interactions of the major factors in determining variation in the parameters of disease severity (Table 7.4).

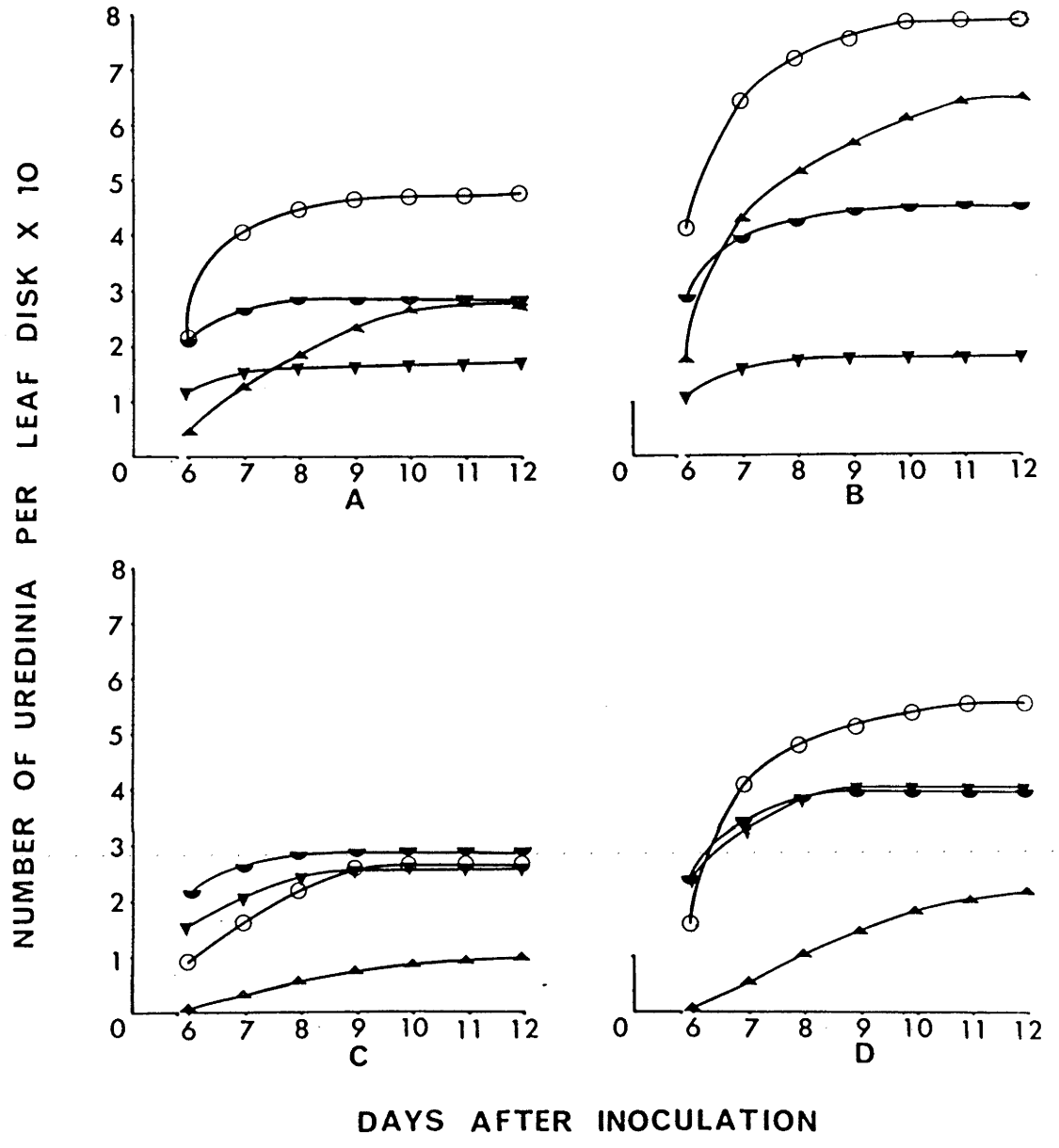

Figure 7 Disease progress curves (ULD) for four cultivars of *Populus* spp. (*P. X euramericana* cvv. *I-214* (▲), *I-488* (▼) and *P. deltoides* cvv. *W 79/304* (◒), *W 79/307* (○) in different combinations of pre- and post-inoculation photoperiod (A, 10/10 B, 10/15 C, 15/10 and D, 15/15h)

With the exceptions of *P. X euramericana* cvv. *I-488* and *I-214* in the combination 15h pre-/15h post-inoculation photoperiod, all the curves for disease progress (basis number of uredinia per leaf disk) in the cultivars differ significantly (P = 0.01) (Figure 7, Appendix 11). Thus, the differences in disease severity (basis ULD) are not artifacts of the time of final observation of ULD. The disease severity parameter, LP50 is also a measure of the rate of uredinial production, thus these curves confirm the differences in LP50 between the cultivars, when these are exposed to the varying regimes of pre- and post-inoculation photoperiod. Further, although the curves for certain cultivars are reasonably distinct when based on ULD, they overlap during part of the incubation period (Figure 7). This explains, in part, the poor correlation between the relative rating of cultivars for resistance on the parameters ULD and LP50.

7.4 DISCUSSION

Increased light intensity (range 100 to 1000 μE $m^{-2}s^{-1}$ during incubation resulted in reduced infection type (Chapter 4) and lower mean disease severity (parameters IPF, LP1 and ULD) in leaf disks of cultivars of *Populus* species infected with races of *M. medusae* (section 4.3, Chapter 4). However, in the present studies, irrespective of the pre-inoculation photoperiod, and based on all parameters, with the exception of LP50, mean disease severity in the disks of the cultivars is highest when these are incubated in a long (15h), rather than a short (10h), post-inoculation photoperiod. This contrast with the demonstrated effect of light intensity suggests

that, for post-inoculation photoperiod at least, the present observations probably result from light duration rather than light intensity (total radiation).

For most parameters mean disease intensity induced in the leaf disks was higher in those exposed to continuing short, rather than continuing long, photoperiod. In general this agrees with observations that short (6h), when compared with long (18h), day predisposed certain cultivars of tomato to *Fusarium* wilt (Foster and Walker, 1947) and similarly that when particular cultivars of tomato were inoculated with *Verticillium albo-atrum* Reinke & Berth. and exposed to short (4h) and long (16h) photoperiod, disease symptom development was more rapid and more pronounced and 96 to 98 per cent of the plants died in the short, when compared with those in the long, photoperiod (Jones *et al.*, 1975). Further the infection type (reaction) of the *Hope* cultivar of wheat to race 21 of *P. graminis* f.sp. *tritici* (Johnson and Newton, 1940), and of the cultivar *Panier* of barley to *P. glumarum* (Bever, 1934) decreased in a long, when compared with that in a short, photoperiod. Unfortunately, in these latter pathosystems the effect of photoperiod on disease severity (quantitative reaction), as distinct from that on infection type (qualitative reaction), is not available.

Comparable studies on the interaction of pre- and post-inoculation photoperiod in determining disease severity induced by a race of a pathogen in cultivars of its host are not reported. Maximum mean disease severity (most parameters) was expressed in leaf disks incubated, in the long, rather than the short, photoperiod (Table 7.1 and 7.3; Figure 7). However, pre-inoculation photoperiod, both as an

independent variable and in interaction with cultivar constitution, was more important than post-inoculation photoperiod in determining variation in disease severity (most parameters - relative values of variance for major factors and their interactions, Table 7.4). While this apparent importance of pre-inoculation photoperiod may be an artifact of the cultivars, race and levels of photoperiods employed, it may be more fundamental, i.e., this importance may be characteristic of hosts which are day-length sensitive and which have indeterminate shoots. Pre-inoculation photoperiod would be expected to affect the 'physiological age' of leaves on such shoots. It has been shown elsewhere that 'ontogenetic' and 'physiological' age of leaves in cultivars of *Populus* spp. affects their susceptibility to *M. larici-populina* (Sharma *et al.*, 1980).

The relative ranking of the cultivars for resistance depends on the parameter employed to measure disease severity (Table 7.1 and 7.2). Again there are minor inconsistencies in the effect of the photoperiod treatments on disease intensity when this is evaluated by different parameters. These observations indicate that the parameters of disease severity employed are not highly correlated in all combinations. It has been reported previously that when complexes of cultivars of *Populus* and races of *M. medusae* are incubated in various combinations of temperature and light intensity the correlations between IPF and LP1 are reasonable and highly significant but the negative correlations between IPF and ULD and LP1 and ULD are very low and often non-significant. These results suggest that environmental variables, temperature and light intensity (Table 4.2 and 4.3, Chapter 4; Table 5.3, Chapter 5; Table 6.2, Chapter 6) or

pre- and post-inoculation photoperiods in the present results, affect differentially the physiological processes leading to these different forms of disease expression.

The parameters employed to measure disease severity are also components of the disease mono-cycle in an epidemic, e.g., reduced IPF, LP1 and LP50 and increased ULD, USM and USU in a disease mono-cycle all increase the value of 'r' (*sensu* van der Plank, 1968) in an epidemic. However, the two- and three-way interactions of the pre- and post-inoculation photoperiods with the cultivar constitution are significant causes of variation in most disease parameters (Table 7.4). These interactions, together with the inconsistent effect of environmental variables on various parameters (section 5.3, Chapter 5; section 6.3, Chapter 6) would make it very difficult to develop for this pathosystem a simulation 'process model' similar to that proposed by Teng *et al.* (1977) for *Puccinia hordei* Otth. on barley.

The artificial nature and fixed model design of the present experiment limit the extrapolation from the present results to other photoperiod, race and cultivar combinations. However, these observations suggest that pre- and post-inoculation photoperiod have the potential to affect disease intensity in *M. medusae* leaf rust in poplar and this possibility should be investigated in the field. Further these and other (Chapter 3 to 6) studies of the effects on this leaf rust of variation in components of the environment all emphasise the interactive nature of the model underlying this host/pathogen/environment relationship. The development of cultivars suited for establishment at lower latitudes is a major aim of poplar breeding in Australia (Pryor and Willing, 1965). It seems likely that

cultivars selected for high relative resistance to this leaf rust in a particular environment, e.g., Canberra latitude 35°S, may not be equally resistant when established elsewhere, e.g., Kempsey or Grafton (New South Wales, latitude 31°S).

CHAPTER 8

TEMPERATURE-LIGHT INTENSITY-PHOTOPERIOD EFFECTS ON THE DEVELOPMENT OF *MELAMPSORA MEDUSAE* LEAF RUST IN CERTAIN CULTIVARS OF *POPULUS* SPECIES.

8.1 INTRODUCTION:

The effect of temperature, light intensity and their interaction on the occurrence and severity of certain rusts have been reviewed in previous chapters (Chapter 4 and 5). However, the interaction of temperature and/or light intensity with the photoperiod of incubation in determining the severity of such diseases has not been reported.

In earlier experiments (Chapter 4 and 5), the temperature and light intensity of incubation, races of *M. medusae* and cultivars of *Populus* spp. were significant contributors to the qualitative and quantitative variation in the development of *M. medusae*, leaf rust of poplar. In addition, most interactions of the above factors of disease development were significant causes of variation in most parameters employed to assess disease severity.

Pre-inoculation temperature (Table 6.2, Chapter 6) and pre-inoculation photoperiod (Table 7.4, Chapter 7) were significant contributors to the severity of leaf rust caused by race(s) of *M. medusae* in cultivars of *Populus* species. However, pre-inoculation temperature interacted significantly with the post-inoculation temperature and the cultivar employed (Table 6.2, Chapter 6).

Similarly, most interactions between pre-inoculation photoperiod, post-inoculation photoperiod and cultivar of *Populus* species were significant (Table 7.4, Chapter 7).

This chapter reports a factorial experiment in which four cultivars of *Populus* spp. were inoculated with urediniospores of race 4 of *M. medusae* and subsequently incubated at combinations of temperature (15 and 25°C), light intensity (100 and 400 μE $m^{-2}s^{-1}$), and photoperiod (10 and 15h) regimes. The possible significance of the results in the epidemiology of the *M. medusae* X *Populus* species system is discussed.

8.2 MATERIALS AND METHODS:

The general procedures for raising poplar plants, selection of leaves, preparation of leaf disks, multiplication of urediniospores of the races of *M. medusae* and inoculation of leaf disks, in a spore settling tower, followed those described in the appropriate sections of Chapter 2.

8.2.1 GROWTH OF POPLAR PLANTS: Four cultivars of *Populus* species (*P. X euramericana* cvv. *I-214*, *I-488*, and *P. deltoides* cvv. *W 79/304*, *W 79/307*) were grown for two months (well established root stocks bearing 2 to 3 buds (section 2.2.2, Chapter 2) ready to sprout at the start of the experiment were used) under controlled temperature (15°C, dark (10h)/25°C day (14h) and light (fluorescent, intensity 300 μE $m^{-2}s^{-1}$, at pot height, 15h photoperiod) conditions in an L. B. phytotron cabinet (section 2.2.3, Chapter 2).

8.2.2 INOCULATION AND INCUBATION: Eight cover glasses, and twenty four leaf disks of each cultivar were arranged randomly on the revolving base of spore settling tower and inoculated using 5mg urediniospores of race 4 (Table 4.1, Chapter 4) of *M. meduseae*. Three inoculated leaf disks of each cultivar selected at random locations from the base of spore settling tower were placed on plastic foam (section 2.6.2, Chapter 2) in separate compartments in 14cm glass Petri plates. This procedure was repeated five times and initially all the inoculated leaf disks were incubated at 20±1°C in dark for 24h. Subsequently fifteen replicate leaf disks of each cultivar were incubated at each combination of temperature (15 and 25±1°C), light intensity (100 and 400 μE $m^{-2}s^{-1}$) and photoperiod (10 and 15h). Procedures to obtain similar incubation conditions are described elsewhere (section 4.2.3, chapter 4; section 7.2.4, Chapter 7). Distribution of leaf disks from each separate inoculation to every combination of temperature, light intensity and photoperiod regime hopefully ensured greater homogeneity of variance. Five cover glasses, one selected at random from each inoculation, were incubated at 15±1°C in dark for 24h to assess the germination potential of the urediniospores deposited on these.

8.2.3 DATA RECORDED: Disease development on individual leaf disks was recorded daily for 10 days and data on disease parameters viz., IPF - incubation period (days) to flecking (flecks are localised chlorotic or necrotic areas, the earliest visible symptoms of disease), LP1 - latent period prior to eruption of the first uredinium/uredinia, LP50

- latent period to production of 50 per cent uredinia of that recorded at 10 days of incubation, ULD - number of uredinia per leaf disk after 10 days of incubation, USM - number of urediniospores produced per mm^2 of leaf disk surface at 10 days of incubation and USU - number of urediniospores produced per uredinium at 10 days of incubation, were computed. Methods for the estimation of LP50, USM and USU have been described elsewhere (sections 2.8.2.3, 2.8.2.5 and 2.8.2.6, respectively, Chapter 2). The inoculated cover glasses were used to compare the deposition density of urediniospores between different inoculations (Appendix 8) and germination potential of these spores (Appendix 9). Deposition of urediniospores on the cover glasses in successive inoculations did not differ significantly (P = 0.01) from that within an inoculation. The germination of the spores exceeded 90 per cent.

8.2.4 STATISTICAL ANALYSIS: The data for all the disease parameters were tested for homoscedasticity and normality (section 2.10, Chapter 2). ULD values were transformed [$\log_e$(ULD+1)] to satisfy these assumptions of the analysis of variance. Analysis of variance was conducted using an SPSS programme (section 2.10.1, Chapter 2). Mean values for each disease parameter for individual combinations of cultivars, temperature, light intensity and photoperiod of incubation were compared by least significant difference (section 2.10.1, Chapter 2).

8.3 RESULTS:

All cultivars, irrespective of the incubation temperature, light intensity and photoperiod regime, developed chlorotic flecks initially but subsequently *P. deltoides* cvv. *W 79/304* and *W 79/307* developed necrotic flecks. Frequently necrosis developed around the uredinia produced on cultivars of *P. deltoides* but not around those on cultivars of *P. X euramericana*. Such necrosis was generally more pronounced on *P. deltoides* cv. *W 79/304* when incubated at 25°C/400 μE $m^{-2}s^{-1}$ /long (15h) photoperiod.

The design of the present experiment permits simultaneous comparisons (P = 0.01) of disease severity (as assessed on six parameters) on four cultivars of *Populus* species inoculated with urediniospores of *M. medusae* and subsequently incubated at combinations of temperature (15 and 25°C), light intensity (100 and 400 μE m^{-2} s^{-1}) and photoperiod (10 and 15h) regimes (Table 8.1).

When averaged across all conditions of incubation then on most parameters (IPF, LP1, ULD and USM) *P. deltoides* cv. *W 79/304* is the most susceptible cultivar. The ranking of other cultivars for relative resistance/susceptibility is irregular and depends on the parameter employed to assess the disease severity (Table 8.2).

When averaged across cultivars, disease severity as assessed on ULD, is highest at combination of 15°C, 100 μE m^{-2} s^{-1} and short or long photoperiod of incubation but lowest at combination of 25°C, 100 μE m^{-2} s^{-1} and long photoperiod of incubation (Table 8.3). Exceptions to these conclusions based on averaged data are common when means for the individual treatment combinations are examined. These exceptions

Table 8.1 Disease severity, assesed on six parmeters[A] , on four cultivars of *Populus* species inoculated with urediniospores of race 4 of *Melampsora medusae* and subsequently incubated in various combinations of temperature, light intensity and photoperiod regimes

Cultivar	Parameter	Temperature 25°C Light intensity μE m^{-2} s^{-1} 400 Photoperiod 10 hour	25°C 400 Photoperiod 15 hour	25°C 100 Photoperiod 10 hour	25°C 100 Photoperiod 15 hour	15°C 400 Photoperiod 10 hour	15°C 400 Photoperiod 15 hour	15°C 100 Photoperiod 10 hour	15°C 100 Photoperiod 15 hour
I-214	IPF	4.00a	3.00c	4.00a	4.00a	4.00a	4.00a	4.00a	4.00a
	LP1	5.73fgh	5.47hij	5.00k	5.20jk	6.87abcde	6.93abcd	7.13ab	7.00abc
	LP50	6.00k	6.00k	6.00k	5.271	8.00cde	8.47bc	8.80ab	7.47defg
	ULD	1.731mn	2.16hijklm	2.67fghi	1.761mn	2.41ghijkl	2.26hijklm	2.84fgh	2.62fghi
	USM	8759ijkl	10417hijk	37169c	8839ijkl	13810hij	12626hijk	35196c	24306ef
	USU	2065efghi	1897fghij	4089a	2509cdef	2196defgh	2237defgh	3428ab	2477cdef
I-488	IPF	4.00a	3.00c	4.00a	3.33b	4.00a	4.00a	4.00a	4.00a
	LP1	5.93fg	5.33ijk	5.47hij	5.27jk	6.60de	6.80bcde	7.07ab	7.13ab
	LP50	6.73hij	6.13jk	6.00k	6.07k	7.40efg	7.87cdef	9.20a	7.33ijk
	ULD	1.69mn	2.22hijklm	1.85klmn	1.991jklmn	2.16hijklm	1.84klmn	2.58fghij	2.45ghijk
	USM	7181jkl	22096efg	11758hijk	12942hijk	16099ghi	7497jkl	26594de	23516efg
	USU	2179defgh	3942a	3044bcd	2971bcd	3484ab	2062efghi	2813bcde	3320abc
79/304	IPF	3.00c	3.00c	3.00c	3.00c	4.00a	4.00a	4.00a	4.00a
	LP1	5.73fgh	5.00k	5.20jk	5.00k	6.53e	6.67cde	7.00abc	7.07ab
	LP50	6.87ghi	6.33ijk	6.20jk	6.00k	7.00gh	7.73def	8.80ab	8.07cd
	ULD	3.76abc	4.12ab	3.82abc	3.21cdef	4.19ab	3.52bcde	4.37a	3.93ab
	USM	41351c	102352a	41272c	7891jkl	95170a	40720c	51846b	17046efg
	USU	1502hijk	2441cdef	1249ijkl	4631m	2388defg	1514ghijk	1115jklm	5341m
79/307	IPF	3.27b	3.00c	3.00c	3.40b	4.00a	4.00a	4.00a	4.00a
	LP1	6.00f	5.73fgh	5.33ijk	5.67fghi	6.87abcde	6.93abcd	7.00abc	7.20a
	LP50	6.87ghi	6.93ghi	6.07k	6.20jk	7.07gh	7.73def	8.47bc	8.07cd
	ULD	1.46n	2.03ijklmn	2.97defg	1.92jklmn	3.60bcd	3.21cdef	3.91ab	3.74abc
	USM	5129kl	7418jkl	5050kl	22881	51610b	33775cd	39220c	9312ijkl
	USU	1101jklm	5631m	4191m	5701m	2219defgh	19470fghij	1264ijkl	321m

[A] disease paramters as described (section 8.2.3, *ibid.*). Values of a particular parameter scored by same letter(s) do not differ significantly at $P = 0.01$.

Table 8.2 Disease severity, assesed on six parmeters [a] , on four cultivars of *Populus* species inoculated with urediniospores of race 4 of *Melampsora medusae* and subsequently incubated in various combinations of temperature, light intensity and photoperiod regimes.

Cultivar	Parameter					
	IPF	LP1	LP50	ULD	USM	USU
I-214	3.88a	6.17b	7.00a	2.31c (12.01)	18891b	2612b
I-488	3.79b	6.20b	7.09 e	2.10c (8.79)	15960c	2977a
W 79/304	3.50d	6.03c	7.13 e	3.87a (58.23)	49706a	1401c
W 79/307	3.58c	6.34a	7.18a	2.86b (29.60)	19225b	1051d

Each value is the mean of observations on 120 leaf disks.

Values of a particular parameter scored by same letter(s) do not differ significantly at $P = 0.01$.

[a] IPF - incubation period (days) to flecking, LP1 - latent period (days) prior to eruption of the first uredinium, LP50 - latent period (days) to record 50% uredinia afte o 10 days' incubation, ULD - values in the parenthese represent actual number of uredinia per leaf disk after 10 days' incubation and others are their $\log_e (ULD + 1)$ transformations, USM - number of urediniospores produced per mm^2 of leaf surface, and USU - number of urediniospores produced per uredinium.

Table 8.3 Disease severity, assesed on six parmeters [a] , on four cultivars of *Populus* species inoculated with urediniospores of race 4 of *Melampsora medusae* and subsequently incubated in various combinations of temperature, light intensity and photoperiod regimes.

Parameter	Temperature 25°C				15°C			
	light intensity $\mu E\ m^{-2}\ s^{-1}$				Light intensity $\mu E\ m^{-2}\ s^{-1}$			
	400		100		400		100	
	Photoperiod 10 hour	Photoperiod 15 hour	Photoperiod 10 hour	Photoperiod 15 hour	Photoperiod 10 hour	Photoperiod 15 hour	Photoperiod 10 hour	Photoperiod 15 hour
IPF	3.57b	3.00d	3.50bc	3.43c	4.00a	4.00a	4.00a	4.00a
LP1	5.85c	5.38d	5.25d	5.29d	6.72b	6.83b	7.05a	7.10a
LP50	6.62c	6.35cd	6.07de	5.89e	7.37b	7.95b	8.82a	7.74b
ULD	2.16c	2.63b	2.83b	2.22c	3.09a	2.71b	3.43a	3.19a
	(17.49)	(29.14)	(25.53)	(12.74)	(32.57)	(23.55)	(41.94)	(34.34)
USM	15605d	35571b	23812c	7990e	44172a	23655c	38214b	18545d
USU	1118c	2211ab	2200ab	1628c	2572a	1940abc	2155ab	1663c

Each value is the mean of observations on 60 leaf disks.
Values of a particular parameter scored by same letter(s) do not differ significantly at $P = 0.01$.

[a] IPF - incubation period (days) to flecking, LP1 - latent period (days) prior to eruption of the first uredinium, LP50 - latent period (days) to record 50% uredinia after 10 days of incubation, ULD - values in the parentheses represent actual number of uredinia at 10 days of incubation and corresponding values are their $\log_e$ (ULD + 1) transformations, USM - numberof urediniospores produced per mm^2 of leaf surface, and USU - number of urediniospores produced per uredinium.

Table 8.4 Analysis of variance of six parameters [a] of disease severity, resulting from the interaction of four cultivars of *Populus* species inoculated with race 4 of *Melampsora medusae* and subsequently incubated at combinations of two temperature, two light intensity and two photoperiod regimes

Source of variation	Parameter					
	IPF	LP1	LP50	ULD	USM	USU
Cultivar (CVV.)	167.12	14.88	1.56ns	142.33	427.87	103.78
Temperature (Temp.)	128.39	1937.02	864.51	94.09	226.29	13.65
Light intensity (L.I.)	45.78	0.55ns	0.84ns	16.33	132.92	18.53
Photoperiod (P.P.)	136.60	3.91	16.15	8.14	88.23	2.28
CVV. X Temp.	167.12	4.93	14.52	19.84	53.37	6.97
CVV. X L.I.	16.78	3.20	3.32	3.79	199.79	17.33
CVV. X P.P.	62.18	3.06	5.14	1.70	22.89	2.73
Temp. X L.I.	45.78	92.99	90.61	4.53	20.82	0.06ns
Temp. X P.P.	136.60	19.81	0.05	3.36ns	252.80	25.77
L.I. X P.P.	85.14	10.33	44.87	12.61	81.82	0.53ns
CVV. X Temp. X L.I.	16.78	0.18ns	7.15	2.87	23.50	4.67
CVV. X Temp. X P.P.	62.18	4.69	1.94ns	1.25ns	54.12	1.16ns
CVV. X L.I. X P.P.	15.77	0.67ns	2.17ns	1.20ns	23.63	1.37ns
Temp. X L.I. X P.P.	85.14	17.67	54.81	21.29	91.77	2.41ns
CVV. X Temp. X L.I. X P.P.	15.77	0.10ns	3.60	0.97ns	80.35	17.39

Residual degrees of freedom for IPF, LP1, LP50 and ULD were 488 and for USM and USU were 64.
Transformed ($\log_e$ (ULD+1) values of ULD were used in the analysis of variance.
ns - variance ratios are non-significant at $P = 0.05$ or less.
* - variance ratios are significant at $P = 0.01$.
** - variance ratios are significant at $P = 0.05$.
Unscored variance ratios are significant at $P = 0.001$.

probably contribute to the significance of interaction terms in the analysis of variance (Table 8.4).

The analysis of variance demonstrated that most of the major factors (cultivar, temperature, light intensity and photoperiod) and their interactions were significant (P = 0.05 or 0.01) (Table 8.4) causes of variation in most disease parameters. In the analysis of variance a fixed effect model was employed and hence following adjustments of the residual variance (section 2.10.1, Chapter 2) the effects of major factors can be discussed. Addition of the variances and degrees of freedom of the interactions to the variance and degrees of freedom of the residual did not affect the significance of major factors in this experiment.

A comparison of variance ratios of the major factors and their interactions permits an evaluation of the relative contribution of each of these to the total variation in disease severity as assessed for each parameter. For instance, temperature was the most significant factor in determining IPF and LP1 while LP50, ULD, USM and USU were most dependant on the cultivar constitution (Table 8.4). Although the variance due to each major factor was generally much higher than that of the interaction terms involving it, in certain cases effects of major factors were not significant but some of their interactions were significant, e.g., the effect of photoperiod on USU and light intensity on LP1 and LP50 (further details presented in Table 8.4).

Disease severity, at comparable light intensity and photoperiod regimes, when assessed on incubation period and latent period is always higher (i.e. shortest IPF, LP1 and LP50) at 25°C than at 15°C.

In contrast, disease severity as assessed using other parameters, viz., ULD, USM and USU is generally lower on leaf disks incubated at 25 rather than 15°C (Table 8.1 and 8.3). The obvious exception was higher USM at 25°C/400 μE $m^{-2}s^{-1}$ /15h photoperiod treatment (Table 8.3) which resulted from high ULD, USM and USU produced on cultivars *I-488* and *W 79/304* when incubated at 25°C/400 μE $m^{-2}s^{-1}$/15h photoperiod rather than at 15°C/400 μE $m^{-2}s^{-1}$/15h photoperiod (Table 8.1).

At 25°C/10h photoperiod, an increase in light intensity from 100 to 400 μE $m^{-2}s^{-1}$ caused a significant increase in IPF, LP1 and LP50 but a significant reduction in ULD, USM and USU (although, at 15°C/10h photoperiod, a similar increase in light intensity increased USM). With an increase in light intensity from 100 to 400 μE $m^{-2}s^{-1}$, at 25°C/15h photoperiod, IPF decreased significantly but ULD, USM and USU increased significantly. Increasing light intensity at 15°C/15h photoperiod caused a decrease in ULD and USM (Table 8.3). Exceptions from these generalizations based on overall means were common when individual treatment combination means were compared (Table 8.1).

8.4 DISCUSSION:

Inoculated leaf disks were kept at 20±1°C in dark for 24h prior to their allocation to different combinations of temperature, light intensity and photoperiod regimes to ensure uniform germination of urediniospores and subsequent penetration and colonisation of the leaf disks by the fungus (Spiers, 1978, Singh, Unpublished data). Thus these results report the effects of the environmental factors

and their interactions on only the post-penetrative phase of a disease mono-cycle. Extrapolation to the field situation should be made with caution because of the artificial nature of the experiments, e.g., use of detached leaf culture method and form of application of the environmental conditions.

Temperature was more significant than light intensity or photoperiod in determining most disease parameters (Table 8.1, 8.3 and 8.4). For comparable levels of light intensity and photoperiod, increasing the temperature of incubation from 15 to 25°C decreased IPF, LP1, LP50 and ULD (Table 8.3). This agrees with the results reported in previous chapters (Table 5.3, Chapter 5; Table 6.2, Chapter 6). In this and the earlier experiments (Chapter 5) with races of *M. medusae* and cultivars of *Populus* species temperature is a more significant contributor than light intensity to variation in most disease parameters. This agrees with the generalised observation (Yarwood, 1965, Dimock, 1967) on other host/pathogen systems that "temperature is more important than light in disease development". However, the present results contrast with studies on *M. larici-populina* and cultivars of *Populus* species where light intensity was more significant contributor than temperature of incubation in determining disease severity as assessed on most parameters (Chandrashekar and Heather, 1982).

Earlier experiments (Chapter 7) at 20±1°C/100 μE $m^{-2}s^{-1}$ showed that the number of uredinia per leaf disk (ULD) at 10h post-inoculation photoperiod was greater than at 15h post-inoculation photoperiod of incubation. A similar trend, in the present experiment at 15 or 25°C with 100 μE $m^{-2}s^{-1}$, occurred. However, at 25°C/400 μE

$m^{-2}s^{-1}$, in contrast to earlier experiments (Chapter 7) USU and USM were higher at 10h than at 15h post-inoculation photoperiod although ULD followed a similar trend. This contrast in behaviour in different experiments could possibly result from the differences in the light intensity and temperature regimes employed and length of the incubation period (10 days in the present experiment as compared to 12 days in the earlier experiments althogh earlier experiments at similar temperature and light conditions; Figure 5.1 and 5.2, Chapter 5; Figure 6.1, Chapter 6; Figure 7, Chapter 7, showed that no significant increase in number of uredinia per leaf disk occurred after 10 days of incubation). In certain environments, the length of incubation period has demonstrated a significant effect on the sporulation of *M. medusae* (Singh, Unpublished data).

The effects of temperature, light intensity, their combinations (Chapter 5), and photoperiod (Chapter 7) on quantitative development of *M. medusae* have been discussed elsewhere. Further discussion here will concentrate on the interaction between cultivar, temperature, light intensity and photoperiod and the relative significance of these in determining the development of *M. medusae* leaf rust.

The present results confirm the findings of previous studies (section 5.3, Chapter 5) that temperature X cultivar interaction is more significant than the interaction between any two other factors of disease development in determining most disease parameters. The cultivar X light intensity, and temperature X light intensity interactions as reported earlier (Table 5.3, Chapter 5) are significant also. In earlier experiments (Chapter 5), irrespective of the race or cultivar employed, increasing light intensity from 100 to

500 μE m^{-2} s^{-1} caused more pronounced reduction in disease severity (basis ULD) in leaf disks incubated at 25 rather than at 15°C. In contrast here at comparable temperature regimes, an increase in light intensity from 100 to 400 μE m^{-2} s^{-1} caused a comparable effect on disease severity of *M. medusae* on three cultivars while the situation was reversed in *P. deltoides* cv. *W 79/304*. This latter contrast could possibly result from difference in the light intensity regimes (100 and 500 in previous as compared to 100 and 400 μE m^{-2} s^{-1} in the present experiments) employed in these experiments and/or the pre-inoculation conditions (compare section 5.2.2, Chapter 5 with section 8.2.2 of this chapter) under which the plants are raised. Pre-inoculation conditions (e.g. temperature (Table 6.2, Chapter 6) and photoperiod (Table 7.4, Chapter 7) are known to affect the reactions of poplar plants to *M. medusae* and they interact significantly with the post-inoculation environmental conditions. Thus in different experiments, even under similar conditions, minor variations in disease development, similar to those reported here, are possible.

The results of present and those of previous experiments (Chapter 4 to 7) clearly demostrate the sensitivity of the occurrence and severity of *M. medusae* leaf rust to many factors of the environment, e.g., pre-inoculation temperature (Table 6.2, Chapter 6) and photoperiod (Table 7.4, Chapter 7), post-inoculation temperature and light intensity (Table 4.2 and 4.3, Chapter 4; Table 5.3, Chapter 5) and post-inoculation photoperiod (Table 7.4, Chapter 7). In addition most interactions between the pre- and post-inoculation conditions (Table 6.2, Chapter 6; Table 7.4, Chapter 7) and within post-inoculation environmental conditions (Table 5.3, Chapter 5) are

significant. Such high sensitivity of this leaf rust to the environmental conditions would suggest that this host pathogen system is naturally (because of the variability of environmental conditions and hence complex relationships in the field) well buffered against the development of epidemics. The present results support the conclusion, drawn from studies on *M. larici-populina* (Chandrashekar and Heather, 1982; Heather and Chandrashekar, 1982) that "*Melampsora* X *Populus* system would be very a very stable system under the natural conditions because of the complex host X pathogen X environment relationships.

The relative resistance of cultivars depended on the environment of incubation and the parameter employed to assess the disease severity. This is analogous to previous studies on this host pathogen relationship (Chapter 4 to 7) and hence prior to recommending cultivars to the growers a rigorous testing of inherent resistance of these under cotrolled environmental conditions in the laboratory is very desirable. A further discussion of the management of these relationships is presented in Chapter 9.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

9.1 INTRODUCTION

A general discussion of the results, reported in the foregoing chapters (3 to 8) and considered to share common underlying basic mechanisms or to lead to similar conclusions about the host/pathogen/environment relationships, is provided in this separate chapter. Broader implications of the results discussed in the previous chapters and their relation to the current opinions and hypotheses advanced for other host/pathogen systems are discussed. While the discussion is speculative at times, hopefully it is developed logically and supported by relevant literature for other host/pathogen systems. A framework for future experiments, based on the information obtained from the current studies, is provided. For clarity of presentation, the subject matter is organised into various sections covering related information.

9.2 LIMITATIONS AND ADVANTAGES OF THE EXPERIMENTAL SYSTEM:

The use of detached leaf culture (Person *et al.*, 1957; Samborski *et al.*, 1958; Browning, 1954), and the selection and form of application of environmental variables in the current studies reflect the artificiality of the experimental system employed. Thus

extrapolations from the present studies to the field situation should be made with caution.

In poplar, resistance to leaf rust varies with leaf maturity and age of the shoot. Young, newly emerged leaves of poplar are immune, the oldest leaves are somewhat susceptible while the leaves of intermediate age, borne on the central portion of the shoot, express maximum susceptibility. In contrast, leaves of a particular maturity are more susceptible to rust in young rather than in older shoots (Sharma *et al.*, 1980). Thus an individual shoot appears to act as an organised unit in determining the relative susceptibility of its component leaves (Chandrashekar, 1981). In the present experiments leaves of same maturity from shoots of the same approximate age were employed to minimise the within sample variation in resistance. This procedure further limits extrapolation from the experimental results to the probable field situation.

Pre-inoculation environment has a significant effect on the reactions of cultivars of poplar to *M. larici-populina* (Chandrashekar and Heather, 1981c) and *M. medusae* (Chapter 6), and it interacts significantly with the post-inoculation environment. This might further limit the extrapolation from results of *in vitro* experiments to the field situation.

In a plant, apparent resistance to a disease can occur due to escape, e.g., the pathogen is geographically isolated from its host or the pathogen is present but fails to establish intimate contact with the susceptible tissue in the host due to unfavourable environmental conditions. The latter type of escape has relevance to the present studies. For example, urediniospores of *M. medusae* do not

germinate at 25°C and 500 μE m^{-2} s^{-1} (Appendix 9) and hence inoculated leaf disk of compatible cultivars (Chapter 4,5,8) when incubated under these conditions would appear to be immune. However, these leaf disks are susceptible under these incubation conditions if this inhibition of germination of urediniospores is overcome (Chapter 4,5,8). To avoid such possibilities and to investigate the effects of the environmental factors on post-penetrative reactions of the cultivars and races, the inoculated leaf disks were pre-treated at environmental conditions congenial for uniform urediniospore germination and subsequent penetration of the leaf disks by the fungus. Such a procedure further restricts the potential for extrapolation from the results. However, this seemed a very appropriate approach in understanding the fundamental host/pathogen/environment relationships (Nelson and Kline, 1963; Hooker, 1967; Dimock, 1967).

The ease of vegetative propagation in poplars (section 2.2.2, Chapter 2) and the multiplication and maintenance of mono-urediniospore (section, 2.4.2, Chapter 2) isolates and assessment of race and cultivar interaction under known environmental condition make this system very useful for studying different aspects of host/pathogen relationships. The exclusion of significant hyperparasites (section 2.9, Chapter 2) is an advantage of the aseptic conditions possible in the system. The system employed is technically sound because the variation in the experiments can be minimised by using a high number of replications (leaf disks), hence increasing the homogeneity (through selection and randomisation) of the leaf disks employed and by employing constant levels of

environmental factors. Consequently in the ANOVA, the value of residual variance is low and the relative independant and interactive effects of the host, pathogen and particular environmental variables can be demonstrated with a greater degree of confidence (Steel and Torrie, 1960).

9.3 HOST/PATHOGEN RELATIONSHIPS

9.3.1 TERMINOLOGY OF THE HOST/PATHOGEN REACTIONS

Terminology of the host/pathogen interactions in plants is very confusing (Federation of the British Plant Pathologists, 1973; Sidhu, 1975; Chandrashekar, 1981) and most of the terms can not be used without reservation. Attempts have been made to minimise this confusion (Robinson, 1971, 1973, 1976; Clifford, 1975; Nelson, 1978; Johnson, 1979) but no satisfactory solution to this problem is achieved. Probably this difficulty results from the complex, variable interactions occurring in various host/pathogen systems. The terms used originally to describe certain relationships in a particular host/pathogen system might not be directly applicable to similar relationships in another system and hence new closely related terms are coined by different workers. In addition, because of the complexity of the mechanisms operating during disease development, various workers, according to their background, use different terms to describe a similar phenomenon. Further nomenculatural complications arise when the host/pathogen reactions, as recorded in the present experiments, are environmentally sensitive (discussed

later). In this thesis widely accepted terms (Horsfall and Dimond, 1959; Federation of the British Plant Pathologists, 1973; Horsfall and Cowling, 1978) are adopted and, if necessary, a brief description of these is given.

9.3.2 THE CONCEPT OF GENE-FOR-GENE RELATIONSHIPS

In certain host/pathogen systems, while resistance in the host and pathogenicity in the pathogen are controlled by their respective genes, they are interdependant for their phenotypic expression (Flor, 1971). Based on parallel inheritance studies of cultivars of Flax (*Linum usitatissimum*) and races of its rust (*Melampsora lini*) in relation to corresponding rust reactions Flor (1942, 1955, 1956) hypothesised that for each gene that conditions reaction in the host there is a corresponding gene that conditions pathogenicity in the pathogen. Each gene in either partner of the host/pathogen system may be identified by its counterpart in the other member of the system.

The exitence of a gene-for-gene relationship in *Populus/M. medusae* system is not established. However, Person (1959) has suggested that gene-for-gene relationships should be the rule rather than the exception in host/pathogen systems. Person (1959) has developed a binomial distribution model to determine the presence of a gene-for-gene relationship, in host/pathogen systems, in which the sexual hybridisation of one or of both the interacting partners is not known. Because of the lack of information on sexual stages of *M. medusae* in Australia, and the long generation time of poplars, Person's model may be useful in determining the genetic base of *M.*

medusae/Populus system. The recognition of a gene-for-gene relationship is very important in understanding some of the concepts and hypotheses regarding the co-evolution of host/pathogen systems.

Investigations on the identification of resistance genes in the host and virulence genes in the pathogen are based on the phenotypic reactions (infection type) of different race/cultivar combinations (Flor, 1956, Person, 1959). The present results have demonstrated that the race/cultivar reactions (including highly resistant reaction e.g. infection type 0 and 1) are modified considerably by incubation temperature and light conditions (Chapter 4). Some examples of similar sensitivity of race/cultivar reactions in other host/pathogen systems to environmental conditions are discussed in Chapter 4. Under such situations the genetic ratios, based on the phenotypic reactions of races and cultivars, of the segregating (F_2) population of the host or the pathogen can vary considerably. This might lead to differing interpretation, of the inheritance of resistance in the host or virulence/aggressiveness in the pathogen. In future experiments on genetic variability in *M. medusae/Populus*, and other host/pathogen systems demonstrating such sensitivity (section 4.1, Chapter 4), the environmental conditions should be specified.

9.3.3. PHYSIOLOGIC SPECIALISATION IN *M. MEDUSAE*

The term 'physiologic specialisation' has been defined as the occurrence of entities within morphologic species that differ from each other in one or more physiologic characters including pathogenicity, biochemical properties, cultural variations, spore

germination and ecologic relationships (Stakman and Levine, 1922; Christensen and Rhodenhiser, 1951; Holton, 1953; Fisher and Holton, 1957; Ainsworth, 1971). Conventionally, in the rusts of cereals and other crop plants qualitative reactions of cultivars have been used in the recognition of physiologic races in the pathogen species. However, quantitative reactions have recently been employed by some research workers in the identification of physiologic races (Clifford and Clothier, 1974). In the present studies both qualitative (Table 4.2 and 4.3, Chapter 4) and quantitative (Table 5.3, Chapter 5) differences in reactions of certain cultivars of poplar to races (mono-urediniospore isolates) of *M. medusae* occurred. This agrees with the observations on *M. larici-populina/Populus* spp. system (Chandrashekar, 1981). These observations suggest the possibility of employing the quantitative reactions of certain compatible cultivars, in addition to the conventionally used qualitative reactions of differential cultivars, in identification of physiologic races. However, such techniques would have to take account of the environmental lability of the race/cultivar reactions in this host/pathogen system (Chapter 5 to 8).

Variation in plant pathogens have generally been attributed to mutation, hybridisation (sexual recombination), heterokaryosis, or parasexualism (somatic hybridisation) (Flor, 1971; McIntosh and Watson, 1982). The pycnial and aecial stages of *M. medusae* are not reported in Australia and hence the sexual sources of variation are possibly non-functional. Thus, major sources of variation here would be mutations or parasexualism. *M. medusae* was originally introduced into Australia, in 1972, and high variation in the initial isolates

which migrated from overseas is another possible explanation for the variation recorded in the present studies.

9.3.4 REACTIONS OF POPLAR CULTIVARS TO *M. MEDUSAE*

A range of qualitative (infection type 0 to 4, Chapter 3 and 4), and quantitative (Chapter 3 and 5), reactions of cultivars of *Populus* species to a field collection and to individual races of *M. medusae* was observed. This agrees with the results of Sharma and Heather (1976b) on this host/pathogen system and those of Chandrashekar and Heather (1980), Chiba (1964), and Taris (1968) for the *M. laricipopulina/Populus* spp. system. Similar variations in qualitative and quantitative reactions of other host/pathogen systems have been reported (Stakman and Harrer, 1957; Hooker, 1967; Clifford and Clothier, 1975; Rodrigues *et al.*, 1975; van der Plank, 1968, 1978; Parlevliet, 1979; Singh, 1979; Ellingboe, 1981). Such diversity in the reactions of cultivars of a species has a considerable importance in breeding for disease resistance, in epidemiology, and in understanding the bio-chemistry of host/pathogen reactions.

9.3.5 HOST/PATHOGEN SPECIFICITY

In the present studies both qualitative (Table 4.1 and 4.3, Chapter 4) and quantitative (*sensu* significant race X cultivar interaction for various parameters of disease in the analysis of variance, Table 5.3, Chapter 5) reactions have clearly demonstrated a differential interaction between the races of *M. medusae* and cultivars of *Populus*

species. Differential interaction between races of other rust species and (differential) cultivars of their host species based on qualitative reactions is a very common feature (van der Plank, 1978; Scott *et al.*, 1979). In contrast, differential interaction based on quantitative race/cultivar reactions is not that commonly demonstrated, although some examples of this type of interaction have recently been reported (Wolfe, 1973; Clifford and Clothier, 1974; Johnson and Taylor, 1976; Martin and Ellingboe, 1976; Parlevliet, 1977; Parlevliet and Zadoks, 1977; Rouse *et al.*, 1980; Ikehashi and Kiyosoma, 1981; Latin *et al.*, 1981). Similar to present studies, Chandrashekar and Heather (1983) reported a significant interaction (both qualitative and quantitative) between races of *M. laricipopulina* and cultivars of *Populus* species.

Differential interaction suggests that the interacting partners, i.e., cultivars and races may be exerting a selection pressure on each other. From theoretical evolutionary considerations, differential interaction is an example of continuous struggle for survival (reproduction) between the host and the pathogen (Person, 1959). The selection pressure of a host on its pathogen has been hypothesised to result in break down of qualitative resistance (Watson and Luig, 1968b; Moore, 1977; Wolfe and Schwarzbach, 1978) in cultivars due to the reproductive advantage (Person, 1959; Person and Mayo, 1974) which new virulent genotypes enjoy in the pathogen population.

P. alba cv. *hickeliana* gave a hypersensitive reaction to a field collection of *M. medusae* made in Canberra area in 1975. It gave a susceptible reaction to a similar collection in the following year,

the latent period was relatively long, e.g., 35 days as compared with 4 to 7 days in the compatible *Aigeiros* cultivars (J. K. Sharma and Heather, 1977). In the present studies a field collection of *M. medusae* made in 1980 was compatible with this cultivar but the latent period was only 8 days (Table 3.1, Chapter 3). Similarly cultivar *75/106*, a hybrid between *P. alba* cv. *morocco* and *P. nigra* cv. *evergreen* gave a hypersensitive reaction to a field collection of *M. medusae* made in 1976 (J. K. Sharma and Heather, 1976b) but a susceptible reaction to a field collection made in 1980 (Willing *et al.* Unpublished data). Heather and Chandrashekar (1983) suggested that this occurred due to adaptation of *M. medusae* to overcome the resistance in these cultivars and that such breakdown of resistance is suggestive of oligogenic resistance possiby on a gene-for-gene base. This hypothesis of Heather and Chandrashekar needs further investigation under cotrolled conditions. Again the possibiltiy of ontogenetic predisposition (Sharma *et al.* 1980) of the host and variable effects of environment of incubation needs to be recognised in such investigations.

The significance of quantitative race/cultivar interactions (Chapter 5) in the susceptible cultivars of *Populus* species, as compared to that associated with qualitative interaction might be relatively less important in disease epidemiology. Adaptation of race 41 E 136 type 3 of *Puccinia striiformis* to compatible cultivar *Maris Huntsman* of wheat is suggested to be the basis of its increased susceptibility to this rust (Johnson *et al.*, 1975). In contrast, the significant quantitative specificity of isolates of *Puccinia hordei* to the cultivar *Julia* of barley (Clifford and Clothier, 1974) is

suggeted to have a negligible epidemiological effect (van der Plank, 1978).

Under similar conditions of incubation, difference between races and cultivars were relatively much smaller when assessed on IPF rather than ULD (Table 5.1, 5.2, Chapter 5) and hence the significant race/cultivar interaction in the analysis of variance for IPF might be relatively less important, epidemiologically, than ULD. Thus the relative potential field significance of an interaction would depend, in part, on the parameter employed to assess the disease.

9.3.6 GENETICS OF HOST/PATHOGEN REACTIONS

Information on the genetics of resistance in *Populus* to *M. medusae* leaf rust, comparable with that for the genetic control of resistance in some field crops, is not available. The collection of such data on poplar is difficult due to the dioecious nature and long generation time (*ca.* 7 years from seed to flowering stage) of poplars. Muhle-Larsen (1963) crossed a resistant cultivar with a susceptible cultivar of the *Aigeiros* section of *Populus* and obtained a simple Mendelian ratio for resistance to susceptible among the F_1 hybrid population exposed to inoculum of *Melampsora*. These results are suggested (Thielges and Adams, 1975) to demonstrate that, resistance is dominant and susceptibility is recessive and the resistance is simply inherited (i.e. controlled by 1 or 2 genes). However, further evaluation of the above results, which were obtained from experiments conducted in the field, might be necessary because the present results have demonstrated that the reactions of cultivars depend on

the race and the environmental conditions employed (Chapter 4,5). Thus, the specification of races and the environmental conditions in these studies seems very important.

In some host/pathogen systems the qualitative resistance (e.g. infection type 0 and 1) seems to be controlled by a single dominant gene, however, this kind of resistance can also be controlled by a recessive gene (Nelson, 1978; Ellingboe, 1981). In contrast, the quantitative resistance (e.g. relatively lower number of uredinia and urediniospores) in cartain compatible cultivars is generally considered to be under the control of polygenes while in certain instances it can be monogenic also (Nelson, 1978; Elingboe, 1981).

The ranking of compatible cultivars of *Populus* species for their resistance to leaf rust varied with the parameters employed to assess the disease severity (Chapter 3 to 8). When data on all the cultivars were included, some of these parameters are very poorly correlated. This, suggests that there might be different genes controlling individual parameters or component processes, within a disease mono-cycle or, alternatively, the functional products of the resistance genes in the host or pathogenicity genes in the pathogen change in quality and/or quantity at different stages (Shaw, 1963) in a disease mono-cycle.

9.3.7 VIRULENCE/AGGRESSIVENESS OF RACES V/S RESISTANCE/SUSCEPTIBILITY OF CULTIVARS

When incubated at 15°C/100 μE $m^{-2}s^{-1}$ race 5 was the most, and race 2 the least, virulent of the races of *M. medusae* employed in an earlier

experiment (Table 4.1, 4.2, Chapter 4). Subsequent results (Table 5.2, Chapter 5) demonstrated that, under these conditions of incubation, race 5 is the most, and race 2 the least, aggressive (basis ULD) of these races. This observation is in complete disagreement with the hypothesis (van der Plank, 1978) that stabilising selection (*sensu* reduced parasitic fitness with increasing virulence of races) is a major factor opposing change in the racial composition of pathogen populations. It supports the suggestion of Parlevliet (1981) that stabilising selection may be a 'useless concept' when applied beyond the confines of new race/new cultivar combinations. Races having a wide virulence spectrum have been reported to dominate in the race population of flax rust in Argentina (Vallega and Antonelli, 1960), oat stem rust in Canada (Martens *et al.*, 1970) and wheat stem rust in Australia (Watson, 1970). The combination of wide virulence spectrum and high aggressiveness in a single race could lead to the possibility of appearance of a super-race.

The breakdown of qualitative resistance (hypersensitivity or immunity) of cultivars in various crop plants due to appearance of virulent races in the pathogen population has led many researchers to believe that such breakdown generally results in severe epidemics (Hooker, 1967; Watson, 1970; Ellingboe, 1981). The results (Chapter 3,5,6,7,8) demonstrate that certain cultivars among the compatible cultivars are more resistant (*sensu* quantitative reactions) than others. The results (Table 5.2, Chapter 5) have also demonstrated varying levels of aggressiveness in races of *M. medusae*. This suggests further that the newly developing virulent races might not

necessarily be highly aggressive. The environmental conditions (Chapter 3 to 8) are significant determinants also of the aggressiveness of a race and susceptibility of a cultivar.

Thus the potential for development of a serious epidemic, subsequent to breakdown of qualitative resistance in a cultivar, would depend on the constitution of the cultivar, the race, and the environmental conditions in a particular area.

9.4 EFFECT OF ENVIRONMENT ON RACE/CULTIVAR REACTIONS

The disease triangle (host-pathogen-environment) and disease square (host-pathogen-environment-man) are the two basic models used to explain the variables of disease induction in the natural and agro-ecosystems, respectively (Robinson, 1976). However, as quoted by Heather and Chandrashekar (1983), the hypotheses on the co-evolution and genetic control of disease development (van der Plank, 1968, 1978; Robinson, 1976) generally emphasise the host X pathogen X man interaction, while largely neglecting the environmental component of these models. Possible reasons for this emphasis have been given and the necessity for models that include environment and its interaction with the other variables of disease, in building more comprehensive hypotheses concerning co-evolution and co-exitence in these systems, has been discussed (Heather and Chandrashekar, 1983).

The sensitivity to temperature and light of the interactions of races of *M. medusae* and cultivars of *Populus* spp., which is an original observation, agrees with that reported for races of *M. larici-populina* on comparable cultivars of poplar (Chandrashekar and

Heather, 1981). Such sensitivity possibly results from changes in the functional-gene-products (Edgar and Lielausis, 1964) in the cultivars and/or the races with changing levels of factors of the environment of incubation. In the present experiments these changes occurred in the post-penetration phase of the relationship and thus are an effect on the host/pathogen interaction. Detailed biochemical investigations would be necessary to examine such changes.

The literature on the effect of temperature on the qualitative reactions, e.g., hypersensitivity of cereal rusts, is contradictory. Following an extensive review of temperature sensitive reactions of rusts of field crops, van der Plank (1978) hypothesised that "If temperature affects resistance, it affects it in the direction of reduced resistance at higher temperature". He has argued that increase in resistance at higher temperature as observed by Rajaram *et al.* (1971) is operative only at supraoptimal (above the optimum for the development of the pathogen) temperature regimes. Similar reactions reported by Gassner and Straib (1932a) were thought by van der Plank to be the result of senescence or some other response to artificial conditions and not a specific response to temperature. In contrast Hyde (1982) has recently reported that the wheat cultivar *Maris Fundin* was resistant at 20°C, partially resistant (mesothetic reaction) at 15°C and compatible at 9°C. He has clearly demonstrated that the resistant reaction at 20°C can not be attributed to this temperature being above the maximum cardinal temperature for this pathogen. He reported that the increase in susceptibility of this cultivar at 9°C does not appear to be the result of leaf senescence as suggested by van der Plank while discussing the results of Gassner

and Straib (1932) on similar cultivars. In the present studies (Chapter 4) the resistance response of certain cultivars of poplar to certain races of *M. medusae* was in agreement with the van der Plank's hypothesis (lower resistance at high temperature). However, there are several instances where the reverse situation applied. The latter increase in resistance (infection type 3 or 4 decreased to 0 or 1) with an increase in temperature from 15 to 25°C (Table 4.2, Chapter 4), is in complete disagreement with van der Plank's hypothesis. *M. medusae* has given compatible reactions (infection type 4) on several cultivars when race/cultivar combinations were incubated at 25°C (Table 4.2, Chapter 4; Table 5.1 and 5.2, Chapter 5). Thus this temperature regime can not be regarded as inappropriate for disease development. Further uninoculated leaf disks, incubated at 15 and 25°C, remained apparently healthy.

The range of race/cultivar reaction types in *Populus/M. medusae* system (Table 4.1 to 4.3, Chapter 4), from immunity (0) to extreme susceptibility (4), is to be expected in a host/pathogen system whose components have co-evolved. While hypersensitivity and qualitative race-specificity are commonly demonstrated by an oligogenic host/pathogen system related on a gene-for-gene basis (Flor, 1971), these reactions are not unequivocal indicators of such systems (Person and Mayo, 1974; Scott *et. al.*, 1978). Qualitative race-specific reactions, e.g. hypersensitivity, are not generally environmentally sensitive (Johnson *et al.* 1967) although examples of such sensitivity have been recognised, e.g., with races of stem rust of wheat in adult plants of wheat varieties (Green and Johnson, 1955; Chapter 4). Possibly, the temperature and light sensitivity of the

race-specific reactions of the *Populus* cultivar/*Melampsora* race system is characteristic of populations of cultivars in which there has been minimal selection or breeding for rust resistance. Such cultivars may contain numerous race-specific resistance genes many of which are operative over different ranges of temperature and light intensity regimes. The infection type of a particular race/cultivar combination could then be very variable at different temperature and light intensity combinations of incubation (Table 4.3, Chapter 4).

The intermediate (infection type 2, 3 and X) race/cultivar reactions at 15°C changed generally to hypersensitive necrotic reaction at 25°C (infection type 1) (Table 4.2, Chapter 4). The effects of plasmagenes are reported to be frequently sensitive to the environmental conditions (Jinks, 1964) and the resistance in cultivars or virulence of the races possessing the corresponding genes might be unstable. Thus as suggested by Johnson *et al.* (1967) for similar reactions in oat rust, the mesothetic race/cultivar reactions recorded in the present studies might be determined by plasmagenes.

In the field, differential sensitivity to the fluctuating environmental conditions could impose a restriction on the selective potential of a particular race (with relatively higher virulence and aggressiveness) and it would tend to maintain variation (by favouring the competitive ability of individual races) to become dominant in a population and thus tend to maintain diversity in the pathogen population. Similarly, especially in the natural mixed stands of numerous host genotypes, the loss of variability due to exclusion of susceptible genotypes (Person, 1959) could possibly be overcome due the differential sensitivity of resistance of cultivars to the

environmental conditions. Thus, differential sensitivity to the environmental factors of the race and cultivar reactions could possibly ease the selection pressure (at least during certain periods) of a particular race on a particular cultivar and *vice versa*. Thus, in addition to genetic variability, the differential sensitivity of race and cultivar reactions to factors of the physical environment could probably be helpful in maintaining a stable relationship between the host and the pathogen.

9.5 ASSESSMENT OF DISEASE SEVERITY

The disease severity of leaf rust resulting from interaction between *M. medusae* and *Populus* depended on the race/cultivar combination employed (section 4.3, Chapter 4; section 5.3, Chapter 5), incubation temperature/light conditions, and the time at which the data were collected (Figure 5.1, 5.2, 5.3, Chapter 5; Figure 6.1, Chapter 6; Figure 7, Chapter 7). The relative ranking of cultivars for resistance depended on the parameter employed to assess disease severity, time of disease assessment and the pre- and post-inoculation temperature and light conditions (Chapter 4 to 8).

The relative ranking of cultivars for resistance depended on the time of disease assessment (Figure 5.1, 5.2, 5.3, Chapter 5). For example, when incubated at 15°C/1000 μE m^{-2} s^{-1} cultivar *W 79/304* was more resistant (basis ULD) than cultivar *W 79/307* at 6 or 7 days of incubation while this situation was reversed at 9 to 14 days of incubation (Figure 5.1). This emphasises the importance of time of disease assessment in comparing the relative resistance of cultivars.

In the field, disease assessment at several times during the season might be essential to rank cultivars for relative resistance.

Disease severity in cultivars of poplar induced by *M. medusae* was assessed employing different disease parameters, e.g., IPF, LP1, LP50, ULD, USU and USM. However, these parameters describe various components within a disease mono-cycle and hence, for comprehensive evaluation of resistance in a cultivar, the recognition of resistance based on several parameters of the type employed here seems important. Such information has a special significance when these parameters are poorly correlated (Chapter 3,5,6).

Under most conditions of incubation, the number of uredinia per leaf disk (ULD) on certain compatible cultivars of *P. deltoides* exceeded that produced on cultivars of *P. X euramericana* (Chapter 3 to 8), although uredinial size in cultivars of the latter species was larger (i.e. higher number of urediniospores per uredinium, Chapter 7 and 8) than those induced in cultivars of the former species. This suggests that the ranking of a cultivar for quantitative resistance is dependant on the parameter employed to assess the disease severity and agrees with the observations of Chandrashekar and Heather (1981) on *M. larici-populina* leaf rust of poplar.

Further, a significant interaction between race, cultivar, temperature and light intensity occurred for various parameters of disease. Pre-inoculation temperature (Chapter 6) and photoperiod (Chapter 7) had a significant effect on the relative ranking of cultivars for resistance. Similar effects of environmental conditions on the severity of *M. larici-populina* leaf rust on cultivars of *Populus* have been reported. These relationships suggest that reliable

prediction of disease in the field would be very difficult. Studies of similar systems, because of the inherent problems due to large number of interactive variables involved, are restricted to one race/ one cultivar combinations (Teng and Close, 1978).

To elaborate further, the present results suggest that a cultivar ranking highly resistant in the field during a particular year might behave differently (e.g. relatively more susceptible) in the following years. Additionally, the resistance of this cultivar might be broken when grown in other areas with congenial environmental conditions and certain virulent/aggressive races. This suggests the great importance of evaluating the resistance of cultivars in the laboratory under a variety of simmulated conditions, approximating those in the projected areas of commercial plantations (further discussion is given in the next section).

9.6 MANAGEMENT OF DISEASE RESISTANCE

Use of resistant cultivars is possibly the cheapest and the safest method of disease control. Cultivars in various crops with varying levels of resistance have been successfully developed through breeding techniques. Some of the important methods of selection and breeding of disease resistant cultivars have been described recently (McIntosh, 1977; Russell, 1978). However, in many instances, the absolute resistance of these cultivars was lost in due course. This has been attributed to the appearance of new physiologic forms of the pathogen as a consequence of the selection pressure imposed by large scale mono-culture of a highly resistant cultivar (immune or

hypersensitive reaction) on existing avirulent pathogen population or simply due to increase in already existing races undected at the time when the resistant cultivar was released.

Qualitative resistance in cultivars of *P. deltoides* to races of *M. medusae* (Sharma and Heather, 1976 Chapter, 4) and *M. larici-populina* (Chandrashekar and Heather, 1980) and quantitative resistance in cultivars of *Populus* to races of *M. larici-populina* (Chandrashekar and Heather, 1980) and *M. medusae* (Chapter 4) are race-specific. Furthermore, it has been suggested that resistance in *Populus* to leaf rust is dependant on one or two dominant genes (Thielges and Adams, 1975). Thus long term success in combating leaf rust in the field, by the selection and propagation of resistant cultivars as mono-cultures, may depend on homeostatic pressure, e.g., stabilising selection (*sensu* van der Plank, 1968), in the pathogen countering selection pressure of the cultivar constitution on the composition of races in the pathogen population (Heather *et al.*, 1980a). Establishment of spatial mixtures, simulating 'multilines', has been suggested as a strategy to avoid such selection pressure (Heather *et al.*, 1980b) (discussed in the following text).

There has been an increasing emphasis on the use of quantitative, poly-genically controlled resistance (Walker, 1967; Watson, 1970; Simons, 1972; Zadoks, 1972; Ellingboe, 1981) in a cultivar which potentially decreases the force of selection pressure by the host cultivar on the pathogen races and consequently help in controlling sudden epidemic occurrence of disease. A cultivar that has long incubation period and latent period, and produces a low number of uredinia and urediniospores per unit leaf area is an ideal

source of quantitative resistance. Certain cultivars e.g. *P. deltoides* cvv. *123A-8* and *123A-11* have shown these charateristics in the present experiments (Chapter, 3). Some other cultivars, although apparently quantitatively resistant on a particular parameter of disease, are highly susceptible when the disease severity is assessed on an other parameter. For example, *P. X euramericana* cv. *I-488* as assessed on ULD seems relatively resistant than other cultivars (Chapter 7) while it ranks highly susceptible when assessed on USU. This suggests that investigations on the genetic control of individual parameters of disease could provide valuable information for incorporating into a single cultivar, through breeding techniques, the individual components of quantitative resistance from different genotypes.

It seems desirable (Heather *et al.*, 1977; Singh and Heather, 1982a) that screening of cultivars for resistance to poplar leaf rust should follow a regulated procedure. Initially, the candidate seedlings or cuttings should be established in seed flats in a glass-house and sprinkled with a collection of urediniospores of the rust(s) collected from a number of cultivars in the projected areas of future establishment. The most resistant cultivars or seedlings should then be propagated (*ca.* six replicates per cultivar) in a nursery and, subsequently, leaf disks taken from these should be screened for resistance in an *in vitro* experiment of the type described herein (Chapter 3 to 8). This screening should preferably involve inoculum of urediniospores of *M. medusae* both from field collections and individual races (section 4.3, Chapter 4). Apart from the function of screening, the use of pure races will yield valuable

information on the virulence and aggressiveness of these isolates of *M. medusae*. The *in vitro* screening will give an accurate measure of the relative inherent resistance of the cultivar genotypes. Preliminary information on growth, stem and crown form, and wood properties can be gathered from the replicate plants of cultivars in the nursery. The cultivars which possess high levels of resistance to rust and other desirable characteristics should then be established in field trials in the areas of projected planting. After some three years of field trials it should be possible to select groups of cultivars which possess desirable features and which are suited to the particular field environments.

The production of new cultivars, screening for rust resistance and other desirable characteristics, and field trials should be an on-going process which allows the release to growers of recommended new groups of cultivars every 3 to 4 years. Over a rotation of *ca.* 15 years this will ensure variability in the resistance genomes in the plantation area and hopefully will avoid major epidemics of leaf rust in the plantings.

9.7 CONCLUSIONS

A brief description of the results obtained in the present investigations is given in the Abstract presented at the beginning of the thesis. The experimental results broadly fulfilled the major objectives of the present investigations (section 1.10, Chapter 1). Some of the more important aspects are highlighted here.

1 Certain cultivars of *Populus* are highly resistant (infection type 0 or 1) to *M. medusae* (Chapter 3 and 4). The resistance of these cultivars can be utilised by suitable breeding techniques. In the future, such cultivars would be useful differentials in identifying previously unrecognised races of *M. medusae* which carry virulence genes, necessary for establishing a compatible relationship.

2 From examination of ten mono-urediniospore isolates, tested on a limited series of cultivars, six races of *M. medusae* were recognised (Chapter 4). This suggests a high degree of physiologic specialisation in *M. medusae*.

3 Quantitative, as distinct from qualitative, reactions can be used as a basis for race-identification (Chapter 5).

4 The occurrence of frequent changes in the relative ranking of cultivars for resistance with environment [post-inoculation temperature/light intensity (Chapter 4,5,6) and temperature/light intensity/photoperiod (Chapter 8), and pre-/post-inoculation temperature (Chapter 6) and photoperiod (Chapter 7)] and the race employed suggests that the resistance of a cultivar should be tested for several years in the areas of proposed establishment before such cultivars are released for commercial plantations.

5 The poor correlation of disease parameters among the cultivars would be a limiting factor in modelling (i.e. regression equation) the resistance of a cultivar incorporating various components in a

disease mono-cycle. The modelling of disease development in a mixed population of different cultivars would be even more difficult.

6 The race-specific reactions (chapter 4 and 5) of cultivars of poplar to races of *M. medusae* suggest a potential for host selection pressure on the racial constitution of a pathogen population. However, there is a significant interaction between the races of *M. medusae*, the cultivars of *Populus* and the environmental factors (pre- and post- inoculation) in determining the disease severity (Chapter 4-8). This suggests that despite the race-specificity, this host/pathogen system may be very stable under natural conditions. The artificial commercial plantations of poplar should be designed to incorporate wide range of resistant (qualitatively and quantitatively) cutivars with varying types and levels of sensitivity to environmental conditions and such senstivity of their reactions to the existing races and environmental conditions should tend to resist abrupt epidemic appearance of leaf rust of poplar.

9.8 AREAS OF FUTURE RESEARCH

At present in Australia, poplars are grown over a very limited area and hence economic factors would limit the extensive research on leaf rust. However, poplar wood has a variety of potential uses (section 1.1, Chapter 1) and an increase in area under poplar cultivation might support some of the areas of future research on poplar rust suggested here.

1 The histological and biochemical mechanisms of qualitative and quantitative resistance reations in differential cultivars of poplar to *M. medusae* can provide a better understanding of these reactions. This can be extended to include several races and different environmental conditions to explain some of the results reported in this thesis.

2 Certain species of *Melampsora* have been cultured axenically on synthetic media (Lane and Shaw, 1974; Riou *et al.*, 1975). Culturing of *M. medusae* on synthetic media might be rewarding in understanding the biochemical basis of racial specialisation in this pathogen.

3 The effect of other environmental factors (not studied here), e.g., humidity and nutrition on host pathogen relationships can be studied. The possibility of interaction of these factors with the factors employed in the present studies can be tested. Such studies would provide better understanding of the epidemiology of *M. medusae*.

4 In the present studies pre-inoculation environment (i.e. the environment under which the plants were raised) had a significant effect on the reactions of poplar cultivars to *M. medusae*. Similarly, the urediniospores of *M. medusae* can be multiplied under different environmental conditions and their potential to induce disease can be compared in subsequent inoculation experiments.

5 Long-term hybridisation programmes to determine the inheritance of rust resistance in poplar would be useful.

6 The source of variation in the fungus (e.g. mutations, adaptations, hybridisation, parasexualism) and inheritance studies on virulence of the pathogen under controlled laboratory conditions would provide better understanding of racial specialisation in *M. medusae*.

7 Simultaneous inheritance studies of resistance in the host (5) and virulence in the pathogen (6) would yield information on the possible gene-for-gene nature of this host/pathogen system.

8 Rust-trap nurseries including differential cultivars of poplar planted in different geographic areas could be useful in surveying the racial composition of *M. medusae* and in detecting the appearance of new races.

9 Several chemicals are effective in controlling *Melampsora* leaf rusts of poplar (Peace, 1962; van der Meiden, 1064; Gojkovic and Vugic, 1966; Menzies and Fullerton, 1974; Spiers, 1974; Filler, 1975; Sheridan *et al.*, 1975). The efficacy of these chemicals on different races of the pathogen prevalent in a particular area can be evaluated and the possibility (Shabi and Yaphet, 1976) of acquiring resistance to chemicals by certain genotypes of the pathogen might be invesitgated.

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APPENDIX I

1a DISTINGUISHING CHARACTERS OF VARIOUS SECTIONS OF THE GENUS *Populus*

Division *Phanerogamae* Subclass *Monochlamydeae*
Subdivision *Angiospermae* Order *Salicales*
Class *Dicotyledoneae* Family *Salicaceae*
Genus *Populus*

SECTION	SUBSECTION	LEAVES	LEAFSTALKS	BUDS	FLOWERS (MALE)	FLOWERS (FEMALE)	FRUIT	NATURAL DISTRIBUTION	EXAMPLES
TURANGA Bge.		very varied	in section rounded or flattened near the blade	pubescent	large red catkins 8 stamens	large green catkins 3-red stigmas	3-valve capsule	Central and western Asia Mediterranean	*Populus euphratica* Oliv.
LEUCE Duby	*Albidae* Dode *white poplar*	on short shoots small, elliptic to round; on long shoots lobed; white tomentose beneath	shortish and round (slightly flattened near the blade)	small, pubescent	catkins 8-10cm 6-10 stamens	catkins 5cm 4 stigmas	2-valve capsule	Northern Hemisphere to 40th parallel	*P. alba* L. *P. alba* L. var *hickeliana* Dode
	Trepidae Dode *Aspen*	round or ovate with non-persistent hairs	flattened	small, adpressed and lustrous, occasionally grayish (*P. grandidentata*	catkins 8-10 cm., 5-20 stamens	catkins 10-12cm 2 stigmas	2 valve capsule		*P. tremula* L.
AIGEIROS Duby *Black Poplars*		deltoid, triangular or rhombic with translucent margins	flattened	lustrous	12-60 stamens	2-4 stigmas	2-4 valve capsule	Europe, Asia and Nth America	P.x *euramericana* (Dode) Guinier cv. 'I-124' *P. nigra* L.
TACAMAHACA Spach. *Balsam Poplars*		ovate, length greater than width, base round; under-surface has metallic luster	in section round	large, viscid, aromatic	15-60 stamens	2-4 stigmas	2-4 valve capsule	East Asia, Nth America	*P. trichocarpa* Torr. and Gray
LEUCOIDES Spach.		large, heart-shaped	in section, round	more viscid, lustrous	12-30 stamens	2-3 stigmas	2-3 valve capsule	Far East, Nth America	*P. ciliata* Wall.

Adapted from:
Anon. 1958. FAO, Rome. p14.

Ib SEXUAL COMPATIBILITY BETWEEN DIFFERENT SECTIONS OF *POPULUS*

The sexual compatibility between different taxonomic sections of *Populus* is illustrated in Figure AI; showing natural compatibility between section Aigeiros and Leucoides, Aigeiros and Tacamahaca. and to a certain degree between Tacamahaca and Leucoides.

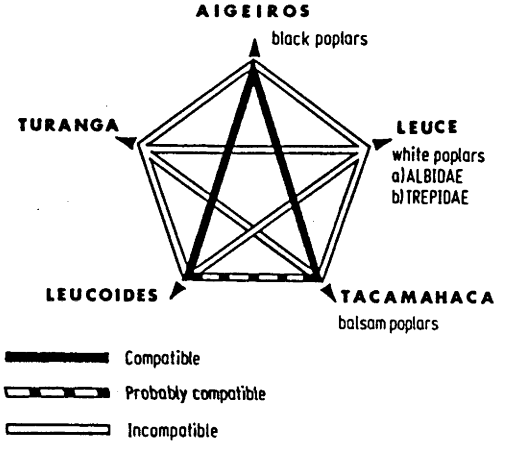


Figure Al. Illustration of cross fertility relationships between various sections of *Populus*. (Reproduced from Willing and Pryor, 1976).

The incompatibility between certain sections of *Populus* have been overcome through a manipulative technique (Willing and Pryor, 1976) which has opened the possibility of crossing certain members (e.g. *P. tremuloides*) of section Leuce, immune to *Melampsora* with certain members (e.g. *P. deltoides*) of section Aigeiros (Knox *et al.*, 1972). This would help in manipulating resistance from taxonomically widely separated sections into desired poplar genotypes.

Ic ORIGIN OF CULTIVARS OF POPLARS USED IN THE PRESENT STUDY

The source and origin of most of the cultivars used in the present experiments have been described elsewhere (section 2.2, Chapter 2 and section 3.2.2.1 and Table 3.4, Chapter 3).

P. X euramericana cvv. *I-214* and *I-488* are hybrids of *P. nigra* X *P. deltoides*. *P. nigra* is of European Origin and *P. deltoides* is of American origin.

APPENDIX II

IIa. PRINCIPLE SPECIES OF *MELAMPSORA* OCCURRING ON *POPULUS* SPECIES.

Species	Distribution	Alternate host	Poplar host
M. occidentalis Jacks	North America	*Pseudotsuga menziesii* *Larix* spp *Pinus* spp	*P. alba*, *P. occidentalis* *P. balsamifera*, *P. trichocarpa*
M. abietis canadensis (Farl.)Arth.	North America	*Tsuga canadensis*	*P. alba*, *P. grandidentata*, *P. balsamifera*, *P. tremuloides*, *P. trichocarpa*
M. larici-populina Kleb.	Europe, Japan South America Australia, New-Zealand, South Africa	*Larix decidua*, *L. leptolepis*, *L. sibirica*	Section Aigeiros and Tacamahaca
M. larici-tremulae	Europe, Japan, South America, New Zealand, South Africa	*Larix decidua*	Section Leuce *P. balsamifera*
M. pinitorqua Kleb.	Europe	*Larix decidua* *L. leptolepis* *Pinus silvestris* *P. pinea* *P. pinaster* *P. halepensis*	Section Leuce
M. alli-populina Kleb.	Europe North Africa	*Allium* spp. *Arum maculatum* *A. italicum* *Muscari cosmosum*	Section Aigeiros
M. rostrupii	Europe, China	*Mercurialis annua*	Section Leuce
M. pulcherrima (Bub) Maire	Mediterranean region	*Mercurialis annua*	Section Leuce
M. magnusiana Wagner	Europe, Asia	*Chelidonium majus* *Fumaria officinalis* *Corydalis* spp.	Section Leuce
M. medusae Thum.	North America, Southern Europe, Australia, New Zealand	*Larix* spp.	Sections Aigeiros and Tacamahaca

(Reproduced from Anon., 1979)

IIb A DETAILED DESCRIPTION OF GEOGRAPHIC DISTRIBUTION OF *M. MEDUSAE*

DISTRIBUTION IN DIFFERENT COUNTRIES: Argentine (Fresa, 1941), Australia (Walker and Hartigan, 1974), Bolivia (Jackson, 1927), Canada (Arthur and Cummins, 1962; Ziller, 1965), France (Dupias, 1943; Taris, 1968), New Zealand (van Kraayenoord, 1974), Papua New Guinea (Heather and Sharma, 1979, Simpson and Arentz, 1979), United States of America (Arthur, 1905) and Urguay (Lindquist and Rosengrutt, 1967).

LOCALISED GEOGRAPHIC DISTRIBUTION OF POPLAR LEAF RUSTS IN AUSTRALASIA:

Locality	*M. medusae*	*M. larici-populina*
Papua New Guinea	+	+
New Zealand	+	+
New South Wales		
Coastal	+	+
Inland	+	+
Queensland	+	+
Victoria	+	+
Tasmania	-	+
South Australia	0	0
Western Australia	-	-

+, Positive record of species ; -, No positive record of species; and 0, Rust present but species not recorded. (Reproduced from Heather and Sharma, 1977).

IIc HOST RANGE OF *M. MEDUSAE*

POPLAR HOST: *P. alba* (Sharma and Heather, 1977), *P. angulata* (Dupias, 1943), *P. balsamifera, P. candicans* (Arthur and Cummins, 1962), *P. deltoides* (Arthur, 1906; 1921; Hills, 1943; Arthur and Cummins, 1962; Toole, 1967; Walker and Hartigan, 1972; McMillan, 1972, 1973; Opie, 1974;), *P. dilatata* (Arthur and Cummins, 1962), *P. X euramericana* (Heather and Sharma, 1977), *P. grandidentata* (Fraser, 1914), *P. nigra* (Walker and Hartigan 1973; Sharma and Heather, 1977; Chandrashekar and Heather, 1981a; 1982), *P. occidentalis* (Arthur and Cummins,1962), P. tremuloides (Arthur, 1909; 1921; Overholts, 1921; Hunt, 1927; 1929; Arthur and Cummins, 1962), *P. wislizeni* (Standley, 1916; Arthur and Cummins, 1962) and *Populus sp.* (Jackson, 1927)

ALTERNATE HOST: *Abies grandis* (Ziller, 1965), *Larix decidua* (Arthur, 1904; 1905; 1906; 1921; Ziller, 1965), *L. laricina* (Arthur, 1906; 1909; 1921; 1934; Ziller, 1965), *L. leptolepis* (Ziller, 1965), *L. occidentalis* (Weir and Hubert, 1917; Ziller, 1965), *Picea sitchensis, Pinus banksiana, P. contorta* (Ziller, 1965), *P. radiata* (Ziller, 1965; Browne, 1968), *P. sylvestris* (Ziller, 1965), *Pseudotsuga menziesii* (Ziller, 1965; Anon., 1967), *Tsuga mertensiana* (Ziller, 1965) and *T. canadensis* (Fraser, 1913).

APPENDIX III

IIIa LIFE-CYCLE OF *MELAMPSORA* LEAF RUST OF POPLAR

The life-cycle of a typical heteroecious, macro-cyclic *Melampsora* species causing leaf rust of poplar is described in Figure A3. The rust organism has two phases, a monokaryotic phase consisting of uninucleate cells, and a dikaryotic phase consisting of binucleate cells. The dikaryotic stages of *M. medusae* causes infection of *Populus* species while the monokaryotic stages infect only the alternate host species (Appendix 2c).

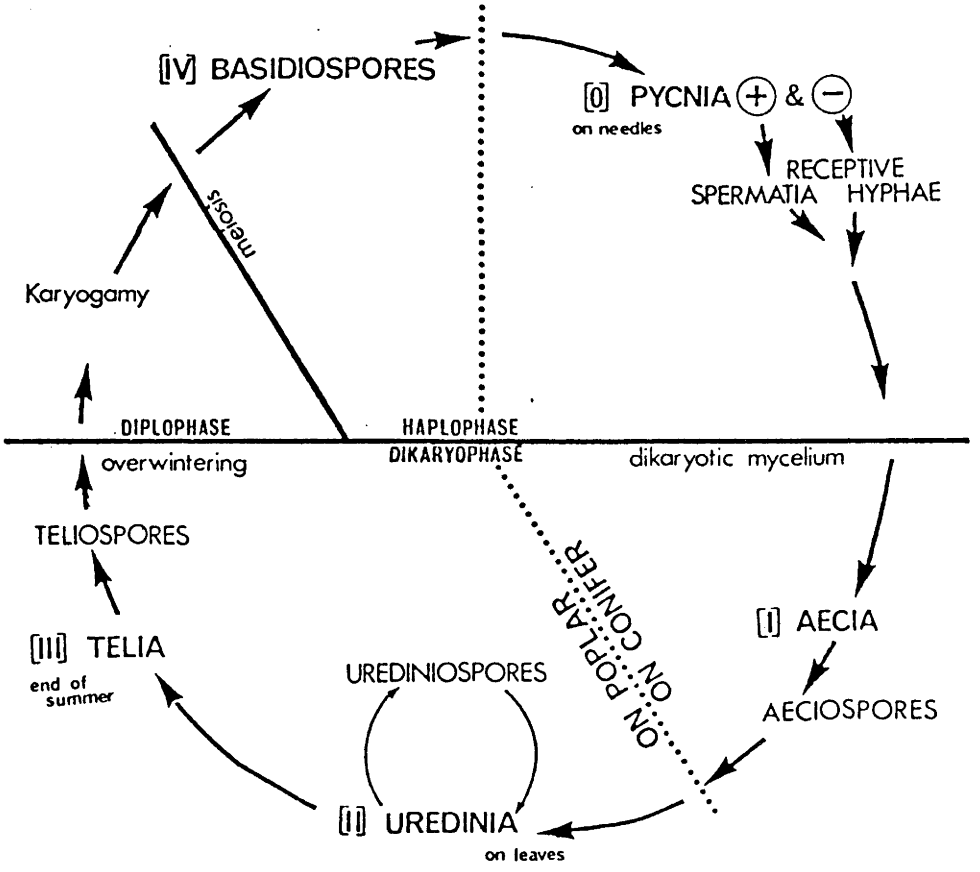


Figure A3 Life cycle of a typical poplar leaf rust
(Reproduced from OMAR, 1978)

IIIb Melampsora medusae: description and specimens examined

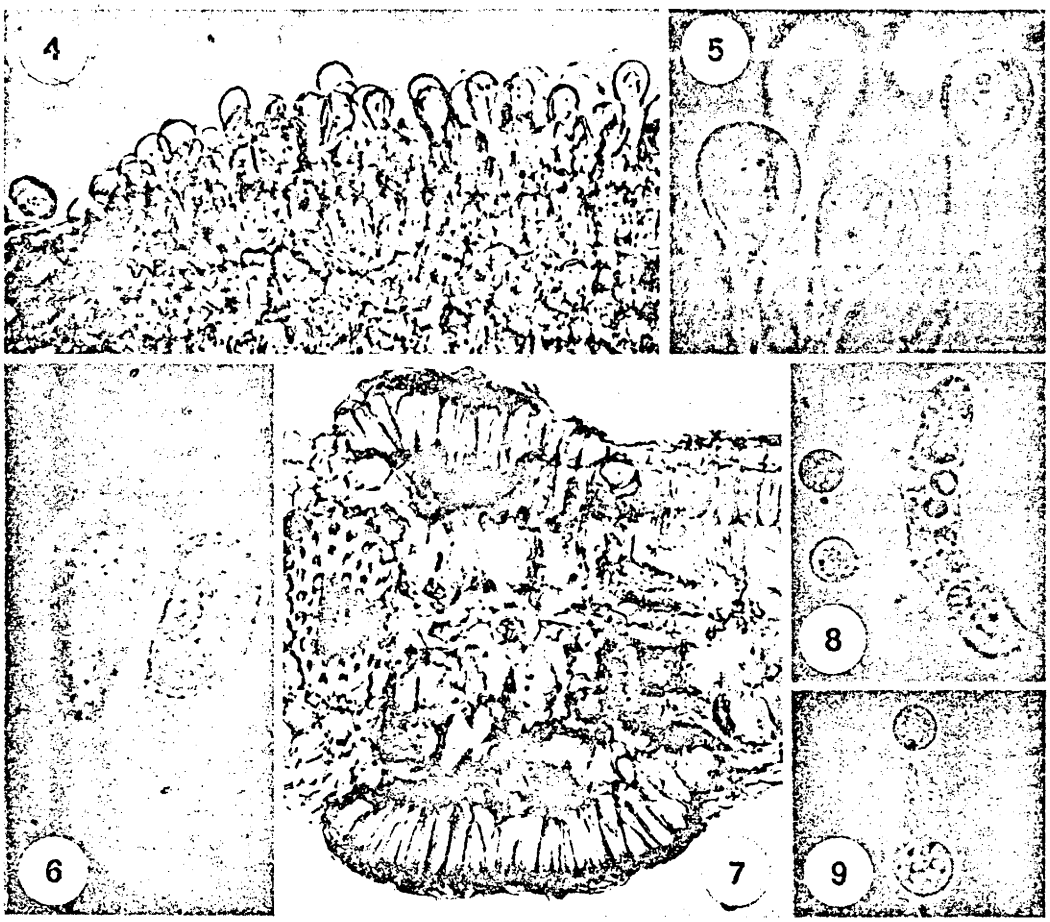
Melampsora medusae Thüm., Bull. Torrey Bot. Club 6, 216, 1878. – *Uredo medusae* (Thüm.) Arth., Result Sci. Congr. Bot. Vienne 338, 1906.

Pycnia and *aecia* not seen. *Uredinia* (Fig. 4) at first subepidermal then erumpent, mainly hypophyllous, a few epiphyllous but smaller and more scattered, golden yellow (Ridgway Cadmium Yellow) when young to orange (Ridgway Orange) when older and in mass, in herbarium specimens drying paler and paling further with age, 150–250 µm diam., very little or no leaf discolouration surrounding sori on most susceptible hosts, some leaf yellowing on less susceptible species, faint pale flecking on upper leaf surface on all hosts. Abundant capitate paraphyses (Figs. 5, 10) present through the sori, to 70 µm long, with stalk 4–6 µm wide and swollen apex which is roughly spherical, (12) 14–17 (19) µm diam. or oval to more rarely clavate 18–22 × 12–16 µm, with a uniformly thickened wall 1.5–3 µm thick, slightly thicker to 4 µm at the apex in a small proportion of paraphyses. *Urediniospores* (Fig. 6. 10) obovate to oval, a few pyriform, with flattened base and rounded apex, (23) 26–35 (37) × 15–19 (21) µm, golden yellow contents, wall colourless, 1–1.5 µm thick above, to 2 µm at the base and thickened on two sides to 3.5–4.5 (5.5) µm. Wall surface echinulate except for a smooth equatorial patch 8–12 µm wide usually extending more than half-way around the spore, rarely as a complete smooth equatorial band; spines 1–1.5 (2.5) µm long, 0.5–0.7 µm diam. and 1.5–3 µm apart, smaller near the smooth patch. Germ pores not observed, but germination seen from 2–4 scattered points on the spore. *Telia* (Fig. 7) on older still hanging leaves mixed with uredinia and abundant on fallen leaves, mainly hypophyllous, some later epiphyllous especially where infection heavy, at first pale amber brown darkening eventually to deep reddish brown (darker than Ridgway Claret Brown or Bay; Munsell 7.5 R 2½3/2) or almost black, darkest when dry, raised slightly above the general leaf surface, subepidermal, roughly circular to irregular in outline, often 3–4 fusing into a larger composite sorus, some sori very small 90–100 µm diam., most larger 250–350 µm diam., the smaller sori often in groups; some telia penetrating the thickness of the leaf and providing teliospores on both surfaces. *Teliospores* roughly cylindrical in side view, circular to angular in cross-section, 30–45 × 11–14 µm, wall pale reddish-brown uniformly 1–1.5 µm thick or occasionally slightly thickened to 2–2.5 µm at the apex, covered by the host epidermis. Reddish-brown material often deposited in host cells above and below telia.

Basidia (Figs. 8, 10) produced as a golden downy layer on surface of overwintered telia, arising from small pore in teliospore apex and borne on a short thin stalk 2–2.5 µm wide and up to 2–3 µm long; basidia cylindric with a rounded apex, straight or slightly curved, 40–65 × 7–8 µm, divided by three transverse septa into four cells, the lowest cell usually slightly longer than the other three; cell contents yellow, wall colourless. Each cell bears a single lateral sterigma 2–2.5 µm wide at the base, 10 µm long and tapering to a fine point; sterigma of apical cell sometimes apical to sub-apical.

Basidiospores spherical, with a minute apiculus where they were attached to the sterigmata, 7.5–11 µm diam., wall colourless, contents pale yellow, often seen to germinate on the overwintered poplar leaf to produce a secondary sterigma 10–12 µm long which bears a slightly smaller secondary basidiospore (Figs. 9, 10). In basidiospore formation almost the entire contents of the basidial cell enters the spore, except for a minute amount remaining in the tip of each sterigma.

Specimens examined. Not all collections examined are listed, but all hosts seen are included. *Populus balsamifera* L., in nursery, Canberra, A.C.T., 20. 3. 1973, A. L. Bertus, DAR 23065, II. *P. canadensis* Moench, Thornleigh, New South Wales, 17. 3. 1972, H. W. Eason, DAR 22958, II. *P. canadensis* "Aurea", Springwood, New South Wales, 26. 3. 1972, A. Searle, DAR 22362, II, III; in nursery, Lysterfield, Victoria, April, 1972, G. Marks, DAR 22097, II, III. *P. deltoides* Marsh., Kempsey, New South Wales, 23. 2. 1972, P. Broadbent and L. R. Fraser, DAR 22963, II; Bathurst, New South Wales, 22. 3. 1972, M. D. Gowland, DAR 22356, II, III; in nursery, Lysterfield, Victoria, April, 1972, G. Marks, DAR 22094, II; Creswick, Victoria, 14. 4. 1972, G. Marks, DAR 22964, II; Mulwala, New South Wales, 9. 5. 1972, J. Walker, DAR 22384, II; Wollongbar, New South Wales, 9. 5. 1972, B. J. Standen, DAR 22381, II, III; Yanco, New South Wales, 21. 6. 1972, R. Bowditch, DAR 22392, II, III; Dundas, 29. 9. 1972, J. Walker, DAR 23003, III, IV; Broadwater, Stanthorpe district, Queensland, 12. 1. 1973, J. Green, DAR 23093 (ex BRIP), II; Brock Park, Stanthorpe, Queensland, 17. 1. 1973, J. B. Heaton, DAR 23095 (ex BRIP), II; Jenolan Caves, New South Wales, 5. 4. 1973, R. Black, DAR 23050a; camping area, Tumut, 16. 5. 1973, J. Walker, DAR 22989, III. *P. deltoides* "Angulata", in plantation, Wright's Creek, New South Wales, 27. 1. 1972, W. Hickling, DAR 22050, II (first Australian record); same locality, 3. 2. 1972, J. Walker, DAR 22053 (portions as DAOM 137840 and IMI 164041), II, III; Corowa, New South Wales, 9. 5. 1972, R. J. Flynn, DAR 22382, II, III; in plantation, Cobrawonga, Victoria, 9. 5. 1972, J. Walker, DAR 22387, II, III; in plantation, Clarencetown, New South Wales, October, 1972, K. Lober, DAR 22998, II; Macksville, New South Wales, 3. 4. 1973, D. Hartigan, DAR 23057, II; in plantation, Tumut, 11. 4. 1973, R. C. Nielsen, DAR 23041, II. *P. deltoides* "Monilifera", in nursery, Lysterfield, Victoria, April 1972, G. Marks, DAR 22095, II. *P. deltoides* cv. Volga, in nursery, Canberra, A.C.T., 27. 4. 1972, K. Eldridge, DAR 22369, II. *P. deltoides* × *trichocarpa* Torr. and Gray, in nursery, Canberra, A.C.T., 27. 4. 1972, K. Eldridge, DAR 22368, II, III. *P.* × *euramericana* I 30, Warkworth, Northland, New



Figs. 4–9. M. medusae. Fig. 4. Uredinium ex DAR 22347, 320 X. – *Fig. 5.* Paraphyses ex DAR 22096, 1000 X. – *Fig. 6.* Urediniospores with smooth central patch ex DAR 22096, 800 X. – *Fig. 7.* Telia ex DAR 22366, 208 X. – *Fig. 8.* Basidium and basidiospores ex DAR 23003, 800 X. – *Fig. 9.* Basidiospore germinating to produce secondary basidiospore ex DAR 23003, 800 X

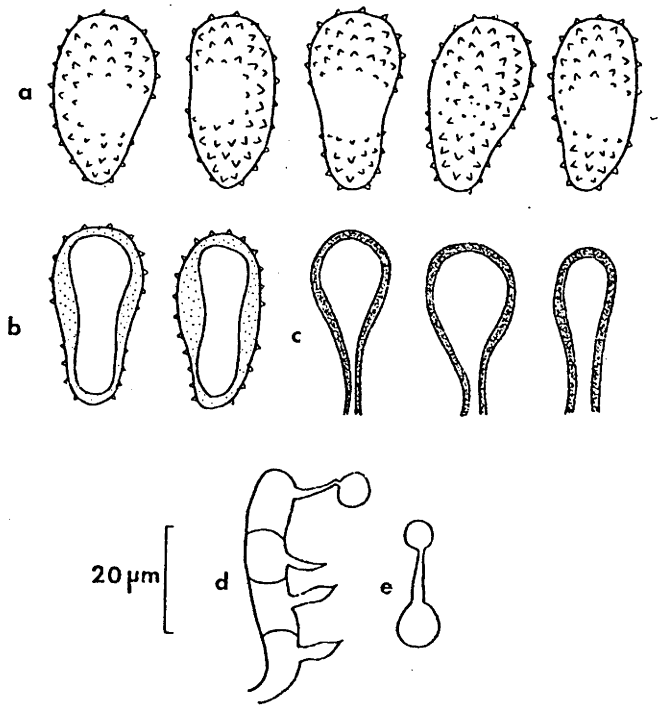


Fig. 10. Drawings of *M. medusae.* a = Urediniospores in surface view; b = Urediniospores in section showing equatorial thickening; c = Paraphyses; d = Basidium; e = Basidiospore germinating

Zealand, April 1973, P. D. GADGIL, DAR 23028, II. *P.* × *euramericana* I 154, Orange, New South Wales, 4. 4. 1973, R. BLACK, DAR 23053, II. *P.* × *euramericana* I 214, in plantation, Tumut, New South Wales, 11. 4. 1973, R. C. NIELSEN, DAR 23044 a, II; Woodhill, Northland, New Zealand, April 1973, P. D. GADGIL, DAR 23027, II. *P.* × *euramericana* I 455, in plantation, Cobrawonga, Victoria, 9. 5. 1972, J. and S. WALKER, DAR 22386, II, III; Whangarei, Auckland Prov., New Zealand, April 1973, J. REGNIER, DAR 22987 (ex LEV 7159), II, III. *P.* × *euramericana* I 488, in plantation, Tumut, 11. 4. 1973, R. C. NIELSEN, DAR 23043, II. *P. nigra* L. hybrid, in nursery, Canberra, A.C.T., 27. 4. 1972, K. ELDRIDGE, DAR 22371, II. *P. nigra* L. var. *italica* du Roi, Oberon, New South Wales, 22. 4. 1973, L. R. FRASER, DAR 23054, II, III; in camping area, Tumut, New South Wales, 16. 5. 1973, J. WALKER, DAR 23013 a, II; 1.5 km west of Braidwood, New South Wales, 17. 5. 1973, J. WALKER, DAR 22991, II, III. *P.* × *robusta* (Simon-Louis) Schneider, in plantation, Cobrawonga, Victoria, 8. 5. 1973, M. JARVIS, DAR 22995, II. *P. simonii* Carr., in nursery, Lysterfield, Victoria, April 1972, G. MARKS, DAR 22096, II. *Populus* × cult. "Evergreen Poplar", Clarencetown, New South Wales, 20. 4. 1972, K. LOBER, DAR 22392, II.

(Reproduced from Walker *et al.*, 1974)

IIIc SOURCE (CULTIVARS) OF PHYSIOLOGIC RACES RECOGNISED IN THE PRESENT STUDY

Mono-urediniospores were selected (section 2.4.2, Chapter 2) from various cultivars of *Populus deltoides* and based on their distinct reaction on differential cultvars of poplar these were reconised as separate races (Chaprter 4).

Mono-urediniospore isolate	Race	Cultivar
A	1	*W 79/1*
B	1	*W 79/2*
C	2	*W 79/2*
D	3	*W 79/7*
E	4	*W 79/7*
F	4	*W 79/12*
G	4	*W 79/12*
H	5	*W 79/13*
I	6	*W 79/25*
J	6	*W 79/25*

APPENDIX IV

THE RATE OF SPREAD OF *M. MEDUSAE* (1972) FOLLOWING ITS INTRODUCTION IN AUSTRALIA

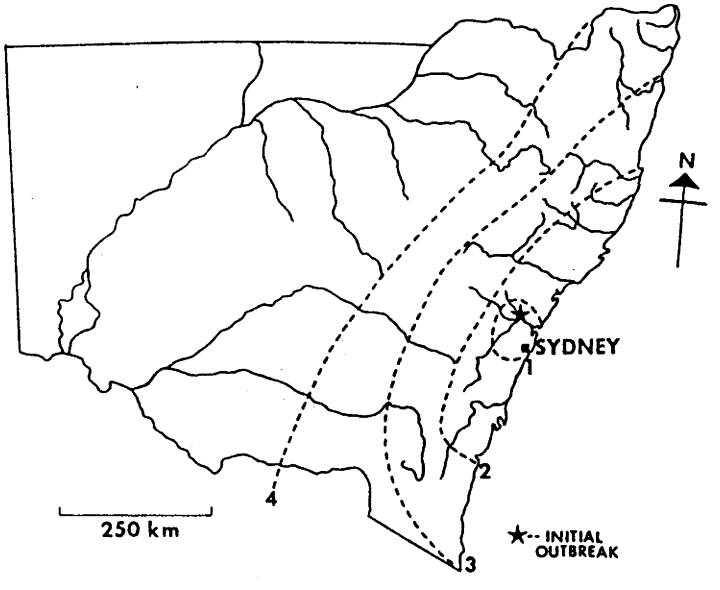


Figure A4 Map of New South Wales showing spread of *M. medusae* after 3, 5, 9 and 14 weeks (Zones 1-4) (Reproduced from Walker *et al.*, 1974)

APPENDIX V

MINERAL COMPOSITION OF AQUASOL

Aquasol is manufactured by Hortico (Private) Limited Australia. It is a nutrient mixture applied at low concentration (ca. 200 ppm) in water to nurseries and potted plants. Its chemical composition is as follows:

Nutrient	Per cent
Zinc (Zn) as Zinc sulphate	0.05
Copper (Cu) as Copper sulphate	0.06
Molybdenum (Mo) as Sodium molybdate	0.0013
Sulphur (S) as Sulphate	0.04
Manganese (Mn) as Manganese sulphate	0.15
Iron (Fe) as Sodium ferric EDTA	0.06
Boron (Bo) as Sodium borate	0.011
Magnesium (Mg) as Magnesium sulphate	0.165
Nitrogen (N) as Mono-ammonium phosphate	1.8
as Potassium nitrate	2.6
as Urea	18.6
TOTAL	23.0
Phosphorus (P) as water soluble Ammonium phosphate	4.0
Potassium (K) as Potassium nitrate	7.8
as Potassium chloride	10.2
TOTAL	18.0
Maximum biuret	0.4

APPENDIX VI

The composition of the nutrient solution based on Hoagland (No. 2) solution (Hewitt, 1966) with some modification to the minor elements is as follows:

Composition		Elements	
$Ca(NO_3)_2.4H_2O$	950 mg/l	N	211.7 mg/l
$(NH_4)H_2PO_4$	120 "	P	32.2 "
KNO_3	610 "	K	235.9 "
$MgSO_4.7H_2O$	490 "	Ca	160.9 "
H_3BO_3	0.6 "	Mg	48.3 "
$MnCl_2.4H_2O$	0.4 "	Na	3.61 "
$ZnSO_4.7H_2O$	0.09 "	S	66.7 "
$CuSO_4.5H_2O$	0.05 "	Cl	0.143 "
H_2MoO_4	0.02 "	Fe	5.007 "
$Co(NO_3)_2.6H_2O$	0.025 "	B	0.105 "
Sequestrene NaFe	62 "	Co	0.005 "
		Mn	0.111 "
NaOH	6.3 "	Cu	0.013 "
		Zn	0.03 "
		Mo	0.012 "

(Reproduced from Anon., 1980).

APPENDIX VII

AN IMPROVED METHOD FOR DETACHED LEAF CULTURE OF *MELAMPSORA* LEAF RUSTS OF *POPULUS* SPECIES.

Culturing obligate fungal pathogens on detached leaves and leaf disks in Petri dishes has been used increasingly following its description by Mains (1917) and assessment of its potential (Clinton and McCormick, 1924; Yarwood, 1934, 1946; Thirumalachar and Narasimhan, 1953). The suitability of this method depends on the type, growth stage and leaf thickness of host plant and the environment (physical and chemical) to which the cut leaves are exposed (Yarwood, 1946; Thirumalachar and Narasimhan, 1953). Excised leaf disks ($1.76cm^2$) have been used to investigate the disease expression by races of *Melampsora* spp. on Poplar cultivars *in vitro* (Sharma and Heather, 1978; Shain and Cornelius, 1979; Chandrashekar and Heather, 1980). In this instance the exclusion of significant hyperparasites (Sharma and Heather, 1978), is an advantage of the aseptic conditions possible in the system.

Circles (14cm diam) of plastic foam were fitted into large Petri dishes and steam sterilized (103KPa, 121°C, 20 min.). After cooling, gibberellic acid solution (10 p.p.m.), sufficient to thoroughly soak the foam, was added to the dishes and the excess drained. Detached leaves of Poplar cultivars were surface sterilized in 0.35% sodium hypochloride solution for 1 min. followed by three washings in distilled water. Employing a spring loaded cork-borer (Figure A), disks ($1.76\ cm^2$) were punched from the leaves under

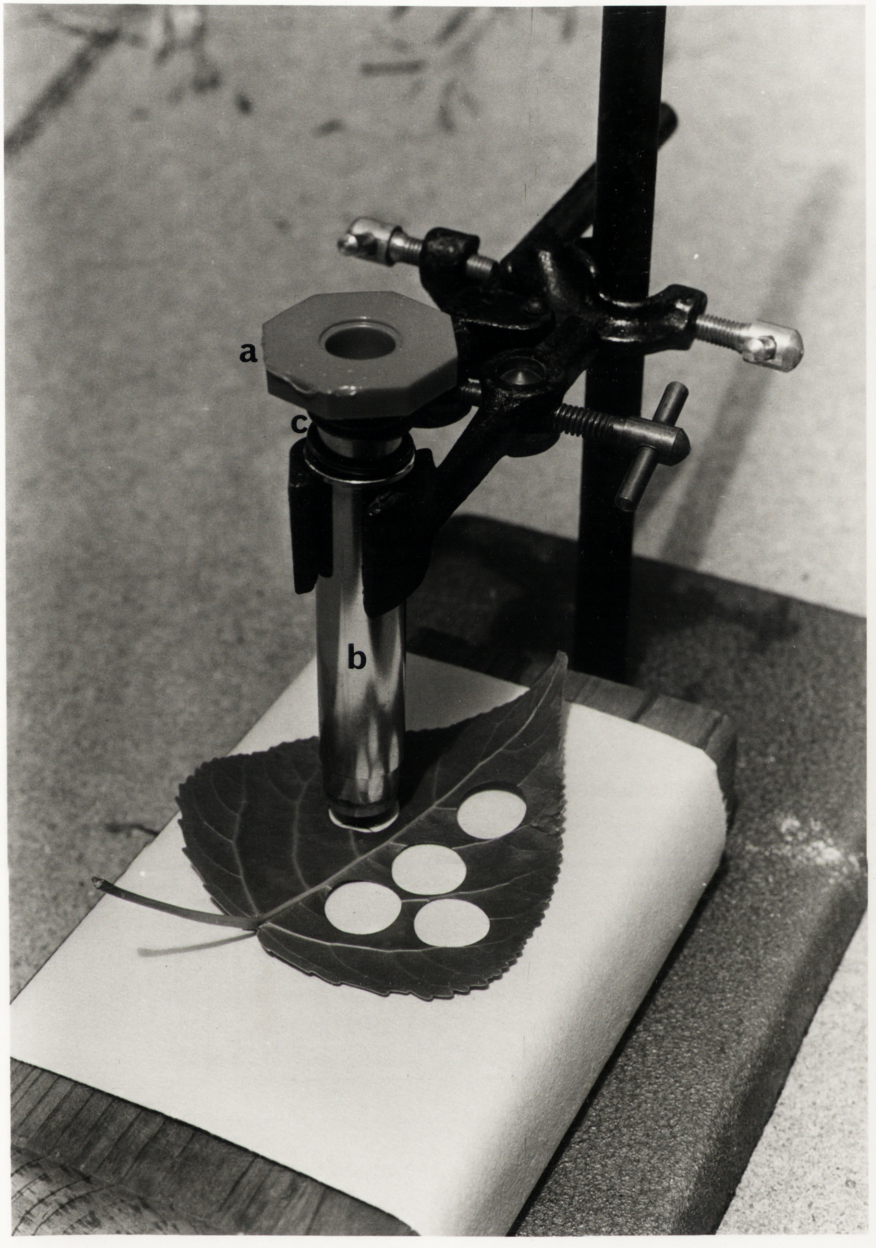


Figure A7 Spring loaded cork-borer. (a) Sliding (inner) cork-borer, (b) fixed (outer) cork-borer, (c) Spring.

sterile conditions. The disks were inoculated on the abaxial surface with contaminant-free, mono-urediniospore cultures of *Melampsora medusae* Thum. in a spore settling tower (Sharma *et al.*, 1980) with a millipore filter (8μ) inserted between the air pump and the spore container. The inoculated leaf disks, abaxial surface uppermost, were transferred on to the foam surface and incubated at 20±1°C under banks of fluorescent tubes (100 μE m^{-2} s^{-1}, photoperiod 16h). Initial symptoms, chlorotic flecks, were recorded on day five. Uninoculated disks remained in an apparently healthy condition for more than 6 weeks, whereas on inoculated disks the disease monocycle was completed normally within 2 weeks. The significant advantages of this method are:

1. The gibberellic acid solution in the Petri doshes is sufficient for 5-6 weeks, thus the opening of the dishes for replenishment during the experiment is unnecessary. The problem of pipetting small quantities into the divided Petri dishes (Chandrashekar and Heather, 1980) is also avoided.

2. The senescence-delaying agent (gibberellic acid) apparently maintains the leaf disks in active condition over long period. This contrasts with the methods using water only (Shain, 1979).

3. The method overcomes the problem of occasional sinking of leaf disks floated on solutions (Chandrashekar and Heather, 1980).

4. The large diameter of the Petri dishes permits adequate replication of leaf disks for studying the multiple cultivar-race reactions under uniform and reproducible conditions.

Petri dishes and plastic foam are recyclable after autoclaving and thus the method is very economical in both materials and labour.

The procedure can be adapted for other host-pathogen systems and is suitable for growth solutions other than gibberellic acid.

(Adapted from Singh and Heather, 1981)

APPENDIX VIII

EXPERIMENTS WITH THE SPORE SETTLING TOWER

Five inoculum doses (3, 5, 10, 15 and 20mg urediniospores) were employed to inoculate (section 2.5.2, Chapter 2) replicate leaf disks of compatible cultivars of *P. X euramericana* and *P. deltoides* (Chapter 5). The inoculated leaf disks were incubated at 20±1°C and 100 μE m^{-2} s^{-1} for 14 days. When the inoculum dose exceeded 5mg, the number of uredinia per leaf disk on cultivars of *P. deltoides* was very high and hard to count because of overcrowding of uredinia. In contrast, when 3mg urediniospores were used, the number of uredinia developed on cultivars of *P. X euramericana* was very low. Thus a uniform dose of 5mg urediniospores was used in each inoculation throughout the experiments because it produced more balanced number of uredinia per leaf disks on cultivars of the two *Populus* species.

The number of urediniospores deposited per unit area was calculated as described by Chandrashekar (1981). The variation in the deposition density of urediniospores within and between the inoculations was less than 10 per cent. In the present experiments on quantitative disease development (Chapter 3, 5 to 8) 10 or 15 replicate leaf disks per treatment were used. This degree of replication proved very satisfactory in minimising the error variance and hence in detecting with greater confidence the significance of effects of major factors and their interactions in determining the level of disease resulting from various race/cultivar/environment combinations (Neter and Wasserman, 1974).

APPENDIX IX

TEMPERATURE-SENSITIVITY OF LIGHT INHIBITION OF UREDINIOSPORE GERMINATION IN *MELAMPSORA MEDUSAE*

ABSTRACT

The germination of urediniospores of *Melampsora medusae*, deposited on cover glasses and incubated in sealed humidity chambers (*ca.* 100%) at 15 and 25°C in darkness and at five levels of increasing light intensity (range 100-500 μE m^{-2} s^{-1}), was recorded at 12 and 24h. At both temperatures, germination (per cent) decreased with increasing light intensity, being a maximum in continuous darkness and zero at 25°C coupled with light intensities of 400 and 500 uE m^{-2} s^{-1}. The latter inhibition of germination was reversible and normal germination occurred when these urediniospores were subsequently incubated in the dark. The inhibitory effect of comparable light intensities was temperature sensitive being more pronounced at 25 than at 15°C.

INTRODUCTION

While reports of the effects of temperature on urediniospore germination are frequent, those for effects of light intensity are much less common (Givan and Bromfield, 1964a,b; Tollenaar and Houston, 1966b). Further, such studies rarely report the effects on germination of concurrent variation in these variables, hence

extrapolation from such laboratory studies to field environments is very restricted.

Spiers (1978) reported that light (intensity and quality unspecified), when compared with continuous darkness, had no apparent effect on the germination of urediniospores of *Melampsora medusae* Thum. Preliminary investigations (Singh, unpublished data) suggested that cool fluorescent light, even at intensities less than 500 μE m^{-2} s^{-1}, was inhibitory to germination of urediniospores of this organism.

This paper reports a factorial experiment in which the effects of concurrent variation in incubation temperature (two levels), light intensity (six levels) and time (two periods) on the germination of urediniospores of *M. medusae* were examined. The temperatures and light intensities employed are within the range of those occurring adjacent to the abaxial surface of leaves of Aigeiros poplars in the field in summer/autumn in southeast Australia.

MATERIALS AND METHODS

PRODUCTION OF UREDINIOSPORES: Leaves of *Populus deltoides* Marsh., bearing apparently contaminant-free uredinia of *M. medusae*, were collected from the field, surface treated with 1% sodium hypochloride for one min, washed twice with sterile distilled water and incubated in closed Petri plates at 20±1°C under cool fluorescent tubes, (100 μE m^{-2} s^{-1} light intensity, 16h photoperiod). After three days, urediniospores were harvested with a spore collecting device, air dried and subsequently deposited in a spore settling tower on surface

treated (as described previously) leaves of *Populus X euramericana* (Dode) Guinier cv. *I-488* following the procedure of Sharma *et al.* (1980). The inoculated leaves were maintained on 10 p.p.m gibberellic acid in closed Petri plates in the temperature and light conditions described previously. After incubation of the inoculated leaves for 11 days, a spore collecting device was used to harvest mature urediniospores which were then discarded. This ensured that urediniospores used in the experiment were all of approximately the same age. The leaves were incubated for a further three days and urediniospores were harvested for use in the germination experiment. The harvested spores were placed in open vials in a desiccator over silica gel at 20±1°C for 12h followed by a further 12h over phosphorous pentoxide. Following desiccation the spores were used immediately in the germination experiments.

GERMINATION STUDIES: Urediniospores were deposited uniformly on cover glasses (13mm diam.), in a spore settling tower. The recommended precautions of Chandrashekar (1981) were observed. The cover glasses were sealed in humidity chambers (Omar and Heather, 1978) with sterile distilled water in the base. This provided a humidity of ca 100% irrespective of external levels of light and temperature. The humidity chambers, enclosed in glass Petri plates, were incubated in two modified phytotron L.B. cabinets (Morse and Evans, 1962). The controls on the cabinets were set to give constant internal temperatures of 15±1 and 25±1°C while a sheet of plate glass below the light box filtered out all that portion of the spectrum below 325 nm. In each cabinet replicate, humidity chambers, enclosed in Petri

plates, were wrapped in several layers of aluminium foil to provide continuous darkness (control). The other humidity chambers were exposed to light intensities of 100, 200, 300, 400, and 500 μE m^{-2} s^{-1} by locating them at various distances in a vertical plane below the light box. Light intensity readings were taken with model LI-170 photometer (Li-Cor, U.S.A.). There were 12 replicate humidity chambers for each light intensity (including darkness) in each L.B. cabinet. Several additional humidity chambers were incubated at 400 and 500 μE m^{-2} s^{-1} in the cabinet at 25°C. Within each cabinet light intensity treatments remained constant throughout the period of the experiment. Six chambers were removed from each light and temperature combination after 12 and a further six after 24h incubation and their cover glasses immediately inverted over a drop of lactophenol on a glass slide. Some of the additional chambers incubated at 25°C and 400 or 500 μE m^{-2} s^{-1} were transferred to darkness at 25°C for a further 24h. Per cent germination for each replicate (cover glass) was calculated from the examination of individual urediniospores (clumps of spores were ignored) in 10 microscopic fields (200 X). A total of *ca.* 180 spores was scored for each treatment. When the length of the longest germ tube was equal to or greater than its breadth a spore was scored as germinated (Manners, 1966).

ANALYSIS OF DATA: For statistical analysis, the mean and standard deviation of transformed (arcsin) per cent germination data were calculated after 12 and 24h incubation at each light/temperature combination. This permitted a comparison of the inhibitory effects of light intensity and temperature on spore germination over time. The

data were tested for homoscedaticity and normality (Neter and Wasserman, 1974) using a GlIM program (Nelder, 1975) and analysed using the sub-program ANOVA of SPSS (Nie *et al.*, 1975). The mean germination values for individual temperature/light combinations were compared by least significant difference (Steel and Torrie, 1960).

RESULTS

Urediniospores failed to germinate when incubated for 24h at 25°C in a light intensity of 400 μE m^{-2} s^{-1} or above (Table A9.1), although occasional spores germinated when the incubation period was extended to 36h. If humidity chambers, previously incubated at 25°C and 400 or 500 μE m^{-2} s^{-1}, for 24h, were placed in darkness at 25°C for an additional 24h then urediniospore germination occurred and was comparable to that of spores held in continuous darkness.

Treatments in which germination was zero were excluded from the analysis of results as their inclusion would have given undue weight to the significance of differences between other treatment combinations. In the ANOVA the second and third order interactions of temperature, light intensity and period of incubation are all highly (P = 0.01) or very highly (P = 0.001) significant (Table A9.2). However, in the analysis a fixed effect model was employed hence, under certain conditions, it is possible to discuss the significance of the individual major factors despite the high significance of the interactions between them. If the total variance due to the interactions is added to that of the residual, and the variance ratios for the independant factors recalculated, then temperature is

Table A9.1 Mean per cent germination (arcsin transformed values) of uredospores of *Melampsora medusae* after 12 and 24h incubation at various combinations of temperature and light intensity.

Light Intensity ($\mu E\ m^{-2}\ s^{-1}$)	Temperature (°C)			
	15		25	
	12h	24 h	12 h	24 h
	Mean*± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
0 (Dark)	78.92ab ± 7.76	83.13a ± 7.54	67.86cde ± 6.78	72.73bc ± 9.21
100	62.98defg ± 2.99	71.53c ± 4.10	53.96h ± 3.35	57.25fgh ± 4.58
200	55.90gh ± 3.69	69.47cd ± 3.48	34.87j ± 4.42	35.39j ± 4.30
300	43.77i ± 5.58	63.81deg ± 4.94	18.54k ± 5.04	20.42k ± 3.57
400	35.81i ± 5.03	62.84defg ± 4.85	00.00 ± 0.00	00.00 ± 0.00
500	35.28i ± 6.00	60.62efg ± 3.47	00.00 ± 0.00	00.00 ± 0.00

Values which share same superscripts do not differ significantly at $P = 0.01$.

*Mean of six replications (approximately 30 urediniospores were observed for each replicate)

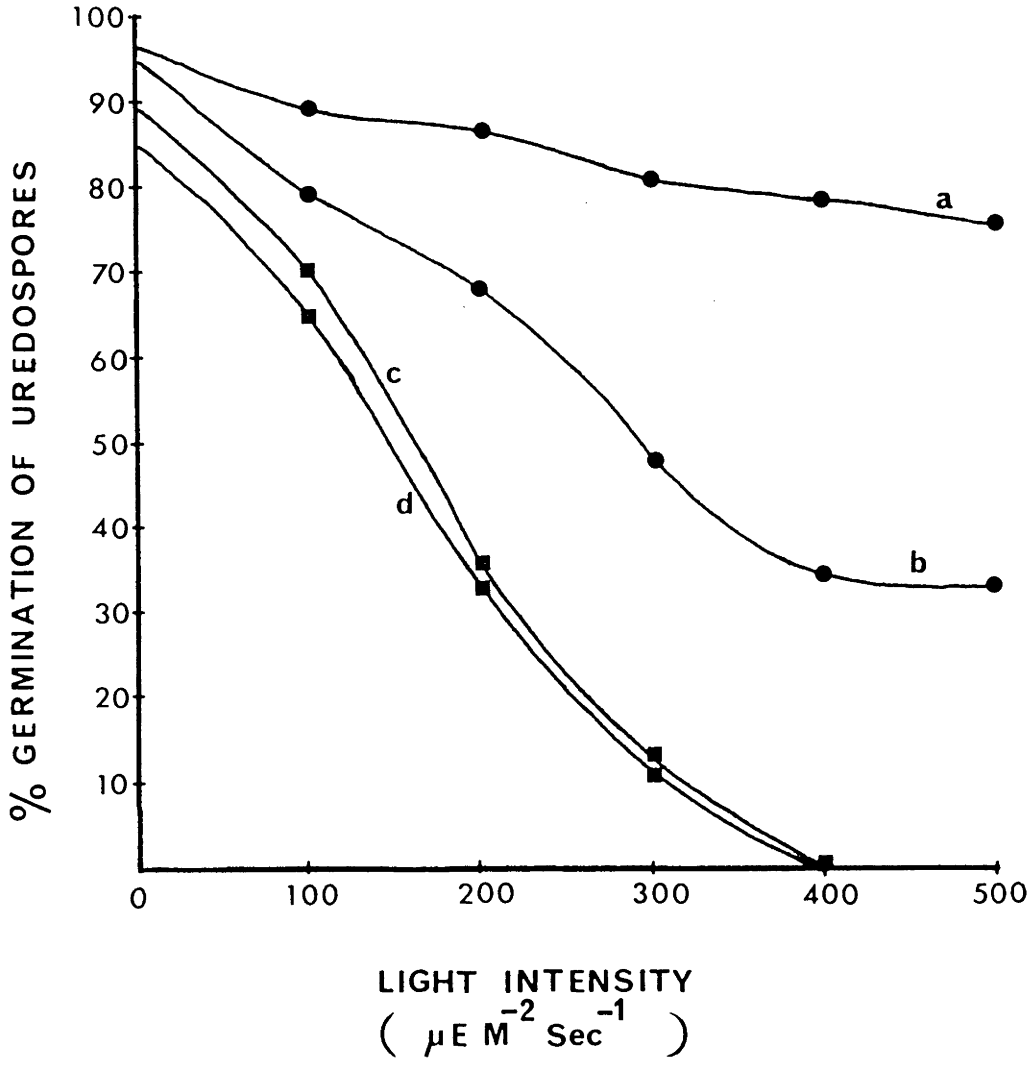


Figure A9 Inhibition of germination of urediniospores of *M. medusae* with increasing light intensity. Symbols: a, urediniospores incubated 24h at 15°C b. urediniospores incubated 12h at 15°C c. urediniospores incubated 24h at 25°C; d. urediniospores incubated 12h at 25°C

Table A9.2 Analysis of variance of effects of temperature, light intensity, period of incubation and their interaction on germination of uredospores of *M. medusae*.

Source of Variation	d.f.	Mean Sum of Squares	Variance Ratio
Temperature	1	32944.065	1431.461
Light	5	10322.867	448.541
Period of incubation (time)	1	2987.534	129.812
Temperature x Light	5	1713.920	74.472
Temperature x Time	1	1944.075	84.472
Light x Time	5	84.846	3.687*
Temperature x Light x Time	5	181.412	7.883
Residual	120	23.014	

*Variance ratio significant at $P = 0.004$.

Variance ratios for all factors * and their interaction (except *) are significant at $P = 0.001$.

the major (P = 0.001) and light intensity a lesser(P = 0.01), contributor to variation in the germination of urediniospores. In contrast the period of incubation is no longer a significant factor in such variation.

For all comparable combinations of light intensity and period of incubation the per cent germination was higher at 15 than at 25°C (Table A9.1). However, in darkness and at a light intensity of 100 μE m^{-2} s^{-1}, the per cent germination after 24h at 25°C did not differ significantly from that after 12h at 15°C; at these light intensities the inhibitory effect of the higher temperature was compensated for by an increased period of incubation. In contrast, at light intensities above 100 μE m^{-2} s^{-1} an increased incubation period of 12h did not compensate for the inhibition of germination occurring at the higher temperature. When urediniospores were incubated at 15°C in light, irrespective of its intensity, germination was always significantly greater after 24 than at 12h. In contrast, when urediniospores were incubated at 25°C in light intensities of 200 μE m^{-2} s^{-1} and above, increasing the period for germination from 12 to 24h did not significantly increase the level of germination.

For the same period of incubation, at either 15 or 25°C, increasing light intensity up to and including 300 μE m^{-2} s^{-1} caused significant reductions in germination of urediniospores. However, increasing the light intensity from 400 to 500 μE m^{-2} s^{-1} at 15° C did not cause a further significant reduction in germination. In contrast to the effect of temperature, incubation for an additional 12h did not compensate completely for the inhibition due to a light intensity of even 100 μE m^{-2} s^{-1}.

The significance of the interaction terms involving incubation period in the ANOVA is accounted for by the full or partial compensating effects which incubation period has on inhibition involving light intensity and temperature. Since this compensatory effect is more pronounced with temperature than with light, the variance of the temperature x time interaction is approximately twenty times that for light x time in the ANOVA. The temperature x light interaction results partly from those effects already mentioned and also from apparent differences in response to increasing doses of light intensity. Increasing light intensity by doses of 100 μE m^{-2} s^{-1} causes a more pronounced inhibition of germination of urediniospores incubated at 25 than at 15°C (Figure A9). However this dosage effect is also partly incubation-period dependent, since when urediniospores were incubated at 15°C, increasing light intensity over the range 100 to 300 μE m^{-2} s^{-1} caused reduced germination when assessed at 12 but not at 24h (Table A9.1). This latter dependance accounts for the significant temperature x light intensity x incubation period interaction in the ANOVA.

DISCUSSION

The temperature during urediniospore production, and the pre-germination exposure of urediniospores to light and the degree of hydration have been shown to affect the subsequent germination of spores of *Puccinia* spp. at varying temperatures and in light and darkness (Givan and Bromfield, 1964a; Tollenaar and Houston, 1966b). Hence, comparison of results obtained in our experiments with those

of other workers with this or other species of the *Uredinales* must be made with caution.

Reduced germination of urediniospores of *M. medusae* at 25°C when compared with that at 15°C agrees with that demonstrated by Toole (1967) for this species and resembles that for *Melampsora larici-populina* Kleb. (Omar, 1978). In contrast, germination of urediniospores of *P. graminis* Pers. f.sp. *avenae* Erikss. & Henn., and *P. coronata* Cda. f.sp. *avenae* Erikss. & Henn. was significantly reduced only at temperatures of 30 and 35°C respectively (Kochman and Brown, 1976a).

In our experiments an increased incubation period of 12h in the dark or at 100 μE m^{-2} s^{-1} compensated, either wholly or partially, for the inhibition of germination occurring at 25°C when compared with 15°C. In contrast, the exposure of urediniospores of *P. graminis avenae* or *P. coronata avenae* at 35°C in the dark for 120 min caused permanent and irreversible inhibition of germination (Kochman and Brown, 1976).

Light inhibition of urediniospore germination in a number of rust fungi has been well established (Givan and Bromfield, 1964a, b; Kochman and Brown, 1976a). In contrast, light has been reported to have no significant effect on (Curtis 1966), or to promote (McCracken and Burleigh, 1962), the germination of spores of *Uromyces phaseoli* Wint. var. *typica* Arth. and *P. striiformis* West., respectively. Our results, demonstrating the inhibitory effect of light, agree with those of Omar (1978) for *M. larici-populina* but contrast with those of Spiers (1978) for *M. medusae*. In the latter instance light (intensity and quality unspecified) did not inhibit the germination

of urediniospores of either *M. medusae* or *M. larici-populina* when compared with that occurring in continuous darkness. Possibly the pre-germination treatment (Tollenaar and Houston, 1966), or racial composition of the urediniospores involved in the experiments, explains the contrast in behaviour.

The reversibility of the inhibition of germination due to higher light intensities demonstrated in our study, agrees with that found for *P. graminis* var. *tritici* (Givan and Bromfield, 1964b). In contrast exposure of urediniospores of *P. graminis avenae* and *P. coronata avenae* to a light intensity of 11250 lux (*ca.* 1000 μE m^{-2} s^{-1}) for 120 min caused permanent and irreversible inhibition of urediniospore germination (Kochman and Brown, 1976).

Light inhibition of urediniospore germination in *P. graminis* var. *tritici* and in *P. recondita* has been shown to be temperature sensitive (Givan and Bromfield, 1964a, b). However, in contrast to present results, the inhibitory effect of light on germination of urediniospores of these two species of *Puccinia* was more pronounced at the lower than at the higher temperature. In the absence of statistical analysis of the data for the *Puccinia* spp. it is not possible to judge whether there was a real interaction between light intensity and temperature similar to that recorded in our results.

Givan and Bromfield (1964a) have hypothesised that the temperature-sensitive nature of the inhibitory response to light intensity suggests enzymatic as well as photochemical reactions. In the ANOVA of our results, light and temperature are independent significant contributors to variability in spore germination; these results support the concept of two reactions each of which is

affected by light or temperature. However, in the ANOVA the light x temperature interaction is also very highly significant. The basis of this interaction is evident if the plots in Figure A9 are regarded as dosage response curves. Thus when incubated at 25°C urediniospore germination demonstrates a typical linear response to increasing doses of light intensity. In contrast when incubated at 15°C, the curves are non-linear at light intensities above 200 μE m^{-2} s^{-1}. This significant light x temperature interaction suggests that both light and temperature affect a particular reaction or alternatively they independently affect individual reactions which are themselves interdependent.

Since light and temperature interact in determining the germination of urediniospores of *M. medusae* they would not be suitable as independent parameters to differentiate races, as has been suggested with certain other rusts (Straib, 1940; Sharp *et al.* 1958; Sood *et al.*, 1966; Sood and Sackeston, 1973).

In the Canberra area, leaf rust of poplar caused by *M. larici-populina* normally occurs in late spring/early summer while that due to *M. medusae* usually commences in late summer. However, the effect of temperature and light intensity on the germination of urediniospores of the two species is very similar. Thus the difference in seasonal occurrence of the two rusts in the field must be due to factors other than those investigated in our experiments.

(Adapted from Singh and Heather, 1982c)

APPENDIX X

Table A10.1 Coefficients of determination† between paired parameters employed to measure the intensity of disease induced on five cultivars of *Populus*, raised on a high and a low pre-inoculation temperature regime, inoculated with an isolate of *M. medusae* and subsequently incubated on a high and a low post-inoculation temperature regime

Pairs of disease parameters	Cultivar				
	I-214	*I-488*	*7-4*	*79/304*	*79/307*
	R^2	R^2	R^2	R^2	R^2
IPF and LP1	0.83	0.93	0.55	0.90	0.13
IPF and LP50	0.87	0.64	0.23	0.29	0.09
IPF and ULD	0.99	0.25	0.49	0.48	0.01
LP1 and LP50	0.97	0.77	0.53	0.57	0.05
LP1 and ULD	0.82	0.50	0.68	0.40	0.37
LP50 and ULD	0.84	0.70	0.57	0.28	0.26

† data pooled across combinations of pre-and post-inoculation temperature treatments.

Table A10.2 Coefficients of determination† between paired parameters employed to measure the intensity of disease induced on five cultivars of *Populus*, raised on a high and a low pre-inoculation temperature regime, inoculated with an isolate of *M. medusae* and subsequently incubated on a high and a low post-inoculation temperature regime

Pairs of disease parameters	Pre- and Post-inoculation temperature (°C) combination			
	Low-low (20/10-20/10)	Low-high (20/10-28/20)	High-low (28/20-20/10)	High-high (28/20-28/20)
	R^2	R^2	R^2	R^2
IPF and LP1	0.48	0.00	0.83	0.11
IPF and LP50	0.60	0.01	0.00	0.00
IPF and ULD	0.20	0.27	0.02	0.01
LP1 and LP50	0.55	0.62	0.10	0.74
LP1 and ULD	0.30	0.13	0.08	0.25
LP50 and ULD	0.00	0.25	0.15	0.65

† data pooled across cultivars.

APPENDIX XI

Table A11.1 Paired analysis of variance of fitted curves for cultivars of *Populus* spp. raised at 10 h pre-inoculation photoperiod and incubated, subsequent to inocualtion with urediniospres of race 4 of *M. medusae*, at 10 h post-inoculation photoperiod regime.

Cultivars Compared	Residual due to	Sum of Squares	d.f.	Mean Square	Variance Ratio
I-214 v/s *I-488*	Deviation from hypothesis	468.9558	4	117.2390	
	Separate regression	0.7442	6	0.1240	945.4754
	Common regression	469.7000	10		
I-214 v/s *79/304*	Deviation from hypothesis	313.0429	4	78.2600	
	Separate regression	0.9571	6	0.1595	490.6629
	Common regression	314.0000	10		
I-214 v/s *79/307*	Deviation from hypothesis	905.9040	4	226.4760	
	Separate regression	12.6960	6	2.1160	107.0302
	Common regression	918.6000	10		
I-488 v/s *79/304*	Deviation from hypothesis	218.4507	4	54.6127	
	Separate regression	0.7493	6	0.1249	437.2512
	Common regression	219.2000	10		
I-488 v/s *79/307*	Deviation from hypothesis	2603.5118	4	650.8780	
	Separate regression	12.4882	6	2.8140	312.7166
	Common regression	2616.0000	10		
79/304 v/s *79/307*	Deviation from hypothesis	1743.2989	4	435.8247	
	Separate regression	12.7011	6	2.1169	205.8781
	Common regression	1756.0000	10		

All variance ratios are significant at $P = 0.01$.

Table A11.2 Paired analysis of variance of fitted curves for cultivars of *Populus* spp. raised at 10 h pre-inoculation photoperiod and incubated, subsequent to inocualtion with urediniospres of race 4 of *M. medusae*, at 15 h post-inoculation photoperiod regime.

Cultivars Compared	Residual due to	Sum of Squares	d.f.	Mean Square	Variance Ratio
I-214 v/s *I-488*	Deviation from hypothesis	2178.0929	4	544.5232	
	Separate regression	2.9071	6	0.4845	1123.8969
	Common regression	2181.0000	10		
I-214 v/s *79/304*	Deviation from hypothesis	666.4940	4	166.6235	
	Separate regression	58.5060	6	9.7510	17.0878
	Common regression	725.4000	10		
I-214 v/s *79/307*	Deviation from hypothesis	2993.6940	4	748.4235	
	Separate regression	18.3060	6	3.0510	245.3043
	Common regression	3012.0000	10		
I-488 v/s *79/304*	Deviation from hypothesis	4701.4989	4	1175.3749	
	Separate regression	56.5011	6	9.4169	124.8155
	Common regression	4758.0000	10		
I-488 v/s *79/307*	Deviation from hypothesis	10243.6989	4	2560.9247	
	Separate regression	16.3011	6	2.7169	942.5907
	Common regression	10260.0000	10		
79/304 v/s *79/307*	Deviation from hypothesis	1291.1000	4	322.7750	
	Separate regression	71.9000	6	11.9833	26.9354
	Common regression	1363.0000	10		

All variance ratios are significant at $P = 0.01$.

Table A11.3 Paired analysis of variance of fitted curves for cultivars of *Populus* spp. raised at 15 h pre-inoculation photoperiod and incubated, subsequent to inocualtion with urediniospres of race 4 of *M. medusae*, at 10 h post-inoculation photoperiod regime.

Cultivars Compared	Residual due to	Sum of Squares	d.f.	Mean Square	Variance Ratio
I-214 v/s *I-488*	Deviation from hypothesis	57.3821	4	14.3455	
	Separate regression	0.4279	6	0.0713	201.1950
	Common regression	57.8100	10		
I-214 v/s *79/304*	Deviation from hypothesis	1545.2671	4	368.3168	
	Separate regression	0.7329	6	0.1222	3161.3484
	Common regression	1546.0000	10		
I-214 v/s *79/307*	Deviation from hypothesis	161.4308	4	40.3577	
	Separate regression	0.5692	6	0.0949	425.2655
	Common regression	162.0000	10		
I-488 v/s *79/304*	Deviation from hypothesis	1031.3890	4	257.8473	
	Separate regression	0.6110	6	0.1018	2532.8806
	Common regression	1032.0000	10		
I-488 v/s *79/307*	Deviation from hypothesis	33.1927	4	8.2982	
	Separate regression	0.4473	6	0.0746	111.2356
	Common regression	33.6400	10		
79/304 v/s *79/307*	Deviation from hypothesis	851.2477	4	212.8119	
	Separate regression	0.7523	6	0.1254	1697.0648
	Common regression	852.0000	10		

All variance ratios are significant at $P = 0.01$.

Table A11.4 Paired analysis of variance of fitted curves for cultivars of *Populus* spp. raised at 15 h pre-inoculation photoperiod and incubated, subsequent to inocualtion with urediniospres of race 4 of *M. medusae*, at 15 h post-inoculation photoperiod regime.

Cultivars Compared	Residual due to	Sum of Squares	d.f.	Mean Square	Variance Ratio
I-214 v/s *I-488*	Deviation from hypothesis	1.3514	4	0.3379	
	Separate regression	0.8136	6	0.1356	2.4915*
	Common regression	2.1650	10		
I-214 v/s *79/304*	Deviation from hypothesis	1981.6777	4	495.4194	
	Separate regression	1.3223	6	0.2204	2247.8195
	Common regression	1983.0000	10		
I-214 v/s *79/307*	Deviation from hypothesis	513.0055	4	128.2514	
	Separate regression	20.2945	6	3.3824	37.9173
	Common regression	533.3000	10		
I-488 v/s *79/304*	Deviation from hypothesis	2025.3531	4	506.3383	
	Separate regression	0.6469	6	0.1078	4697.0155
	Common regression	2026.0000	10		
I-488 v/s *79/307*	Deviation from hypothesis	474.1809	4	118.5452	
	Separate regression	19.6191	6	3.2699	36.2535
	Common regression	493.8000	10		
79/304 v/s *79/307*	Deviation from hypothesis	3958.0722	4	989.7181	
	Separate regression	20.1278	6	3.3546	295.0331
	Common regression	3979.0000	10		

All variance ratios (except that scored by *) are significant at $P = 0.01$.